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A STATISTICAL REPORT ON 955 CASES OF CANCER OF THE CERVIX UTERI AND 321 CASES OF CANCER OF THE CORPUS UTERI.

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In 1938–39 the Clinical Cancer Research Committee of the British Empire Cancer Campaign carried out a clinical survey of all cases of cancer seen in the hospitals, both Voluntary and L.C.C., in the Administrative County of London. This was done by means of questionnaires, one of which was filled in by the Registrars in the various hospitals for each patient and returned to the Clinical Cancer Research Committee for record. The work was interrupted by the outbreak of war after it had been in progress for 17 months. By this time 15,203 cases of cancer had been registered, of which 955 were cases of cancer of the cervix uteri and 321 of the corpus uteri. These patients have now been followed up for five years or more and the records analysed.

PART I: CANCER OF THE CERVIX UTERI.

There were 859 primary and 96 recurrent cases.

Primary Cases.

Civil State.

	No.		Per cent.
Single	37		4·3
Married	644	819	95·3
Widowed	175		
Not stated	3		0·4

Lane-Claypon and McCullagh (1927) give the civil states of 815 women suffering from cancer of the cervix as 3·2 per cent single and 96·8 per cent married or widowed.

Age Distribution.

Age groups.	Single.	Married and widowed.	Not stated.	Total.
25–34	4	22	—	26
35–39	1	45	1	47
40–44	8	85	—	93
45–49	4	129	—	133
50–54	5	147	—	152
55–59	8	145	—	153
60–64	—	98	1	99
65–69	2	75	—	77
70–74	4	47	—	51
75–79	1	19	—	20
80–	—	7	1	8
Mean age	51·5±2·09	54·7±0·37	—	54·6±0·37
Standard deviation	12·7±1·48	10·7±0·26	—	10·9±0·26

This table, together with the corresponding one for cases of the corpus uteri (p. 454), was submitted to Dr. Percy Stocks, Medical Statistical Officer, General Register Office, for his opinion on the statistical significance of the figures. He comments as follows :

" The incidence of cancer of the uterine cervix and corpus uteri according to civil condition and age can best be studied by comparing the ratios of married and widowed to single women amongst the patients suffering from each form of cancer with that to be expected amongst living women on the one hand and amongst women dying of all causes on the other, at separate age groups.

" *Ratios of Married and Widowed to Single Women.*

Age group.	Cancer of cervix cases 1938–9.	Cancer of corpus uteri cases 1938–9.	Living women (estimated).	All women dying in 1938.
25–	5·5	Indeterminate	2·4	2·0
35–	14·4	2·0	4·1	3·6
45–	30·7	3·2	4·8	4·6
55–	30·4	3·3	5·2	5·6
65–	21·1	4·0	4·9	5·6

" Provided that it can be assumed that the hospital sample contains the same proportions of single, married and widowed women as the general population at each age period (or that the preponderance of one or other group is constant throughout the age scale), the following conclusions can be drawn :

" (1) Liability to cancer of the cervix uteri is greater at every age amongst married and widowed than amongst single women, and especially between the ages of 45 and 65, when it is about 7 times as great.

" (2) Liability to cancer of the corpus uteri is somewhat less amongst married and widowed than amongst single women, at any rate between the ages of 35 and 65.

" When the age distributions of the cervix and corpus uteri patients are compared, the percentage distributions with their standard errors are as follows :

	Single women.			Married or widowed.		
	Cervix.	Corpus.	Difference and standard error.	Cervix.	Corpus.	Difference and standard error.
Under 45 .	35·1±7·8	9·1±3·5	26·0±8·6	18·6±1·4	7·7±1·8	10·9±2·2
45–64 .	45·9±8·2	68·2±5·7	22·3±10·0	63·4±1·7	65·4±1·7	2·9±3·6
65 and over	19·0±6·4	22·7±5·15	3·7±8·2	18·1±1·3	27·0±3·0	8·9±3·3
All ages .	100·0	100·0		100·1	100·0	

" Amongst single women the proportion under 45 years of age was significantly greater for cervix than corpus, with a corresponding deficiency at ages 45–65. Amongst married or widowed women the proportion under 45 was again significantly greater for cervix than corpus, but over 65 the proportion with carcinoma of the corpus was significantly greater—27·0 to 18·1."

Heredity.

	No.	Per cent.
Family history of cancer . . .	90	10·5±1·04
" " " in more than one relative	8	1·1
Family history of cancer of the uterus .	13	1·5
No family history of cancer . .	543	63·2±1·6
Not stated	226	26·3±1·5

It was not possible in taking the history to decide whether cancer of the uterus in a relative affected the cervix or the corpus uteri.

The family histories of the 90 patients who gave a history of cancer in the family were examined in more detail, only parents and siblings being included.

	No.	(1) Per cent of all patients of known family history.	(2) Per cent of all patients.
Father suffered from cancer of any other site	24	3·8±0·77	2·8±0·6
Mother suffered from cancer of any other site	34	6·8±1·0	5·0±0·7
Mother suffered from cancer of uterus . .	9		
Brother(s) suffered from cancer of any other site	10	1·6±0·5	1·2±0·4
Sister(s) suffered from cancer of any other site	18	3·5±0·73	2·6±0·54
Sister(s) suffered from cancer of cervix uteri .	4		

The percentages give the high and low values, with the 226 patients for whom no family history was obtained excluded from Column 1 and included in Column 2. There were three instances of both parents having died of cancer among the 631 patients whose family history was known, or 1 in 210. The expectation of

this occurring by chance in this series was 1 in 386. Stocks and Karn (1933) found the expected frequency of this event for cancer of all regions to be 1 in 180, the numbers involved being 364 fathers and 373 mothers. There is, therefore, no indication that heredity has any influence on the incidence of this disease. These figures may be compared with the corresponding ones for cancer of the breast (Harnett, 1948), all of which are almost exactly double those for the cervix uteri, with the same percentage of " not stated."

Relevant Past History.

Menstrual cycle.

Questions were put as to length of interval between the periods, duration of flow, amount lost, and the occurrence of excessive pain. The questions were answered by 84 to 86 per cent of the patients, and the resulting figures were :

		No.	Per cent.
Interval :	Short or irregular	23	2·7
	Normal	704	82·0
	Prolonged, or abnormal but irregular	6	0·7
	Not stated	126	14·7
Duration :	Short, up to 3 days	132	15·4
	Normal, 4–6 days	455	53·0
	Prolonged, over 6 days	144	16·8
	Not stated	128	14·9
Amount of flow :	Scanty	98	11·4
	Normal	485	56·5
	Profuse	131	15·3
	Not stated	145	16·9
Pain :	None or normal in amount	652	75·9
	Excessive at all times or before marriage only	64	7·5
Menstrual cycle not stated		143	16·6

The percentage of deviation from normal in the amount of the flow is greater than Lane-Claypon (1926) found in a control series of 509 women, but those for intervals and duration are much the same.

The menopause.

The relationship of the onset of the disease to the menopause and the age at which the latter occurred were recorded separately for the single women and for the married and widowed.

	Single.	Married and widowed.	Not stated.	Total. No.	Total. Per cent.
Menopause not yet reached	14	277	1	292	34·0
,, was artificially induced	2	17	—	19	2·2
Patient was past the menopause	13	479	1	493	57·4
Not stated	8	46	1	55	6·4

Age at Menopause.	Single.	Married and widowed	Not stated.	Total. No.	Total. Per cent.
Under 40 years	1	22	—	23	4·5
40–	3	63	—	66	12·9
44–	2	148	—	150	29·3
48–	7	175	—	182	35·5
52–	2	78	1	81	15·8
56 and over	—	10	—	10	2·0
Interval between the menopause and onset of symptoms.					
Under 5 years	5	132	—	137	26·8
5–10 years	3	97	1	101	19·7
10 and over	7	267	—	274	53·5
Total number who were past the menopause	15	496	1	512	100·0

The menopause had been artificially induced by sub-total hysterectomy or bilateral oöphorectomy in 15 patients, by radium in 1, and by X-rays in 3 for the treatment of menorrhagia due to fibroids. Lane-Claypon (1926) found that 29 of 509 control patients (5·7 per cent) and 15 of 508 patients with cancer of the breast had had an artificial menopause induced by operation for conditions other than cancer.

Children and miscarriages.	Single.	Married and widowed.	Not stated.	Total.
No children	19	71	1	91
1 child	7	114	—	121
2 children	1	132	—	133
3 ,,	2	98	1	101
4 ,,	—	91	—	91
5 or more children	—	281	—	281
Not stated	8	32	1	41
No miscarriages	25	499	2	526
1 miscarriage	1	137	—	138
2 miscarriages	—	49	—	49
3 or more miscarriages	—	43	—	43
Not stated	11	91	1	103

The 91 nulliparous patients were divided into those who were totally barren and those who had had one or more miscarriages :

	Single.	Married and widowed.	Not stated.	Total.
No children, no miscarriages	18	49	1	68
No children, but one or more miscarriages	1	15	—	16
Not stated	—	7	—	7
Total	19	71	1	91

Of 818 women of known parity (859, less 41 unknown), 91 or 11·1 per cent were nulliparous and 68 or 8·3 per cent were completely sterile. Lane-Claypon (1927) found that 9·3 per cent of 375 women with cancer of the cervix were sterile.

Dr. Stocks comments as follows on these figures when compared with those for corpus uteri (Part II, page 456):

"There were 71 married and widowed women who had not had a child out of 787 of known parity with cancer of the cervix. The expected number, calculated by multiplying the numbers in each age group by the percentages of married and widowed women who were recorded as having had no child at the registration of deaths from all causes in England and Wales in 1939, was 135. There was, therefore, a pronounced deficiency of childless women amongst those suffering from cancer of the cervix. In sharp contrast there were 44 married and widowed women who had not had a child out of 202 of known parity with cancer of the corpus uteri, the expected number by the same method of calculation being only 33."

Interval between last pregnancy and first symptom of cancer in 818 *patients of known parity.*

No children or miscarriages	68
Pregnant at the time	2
Less than 1 year	5
1–5 years ago	26
5–10 ,,	51
10–15 ,,	108
15–20 ,,	95
20–25 ,,	126
25–30 ,,	87
30–35 ,,	60
35–40 ,,	39
Over 40 years ago	37
Not stated	114

The period of maximum incidence was 10–25 years after the birth of the last child. One patient was about 5 months pregnant when the cancer was discovered during examination; she was treated by Caesarian section and Wertheim's hysterectomy, but died from recurrence 18 months later. Another patient passed some pieces of tissue whilst in labour which were found to be carcinomatous; she was treated by radium and X-rays, but died from recurrence 18 months later.

History of instrumental deliveries.	No.	Per cent.
No children or miscarriages	68	7·9
Has had one instrumental delivery	121	14·1
,, more than one instrumental delivery	36	4·2
None	549	63·9
Not stated	85	9·9

History of previous operations.	No.	Per cent.
Cervix operation	29	3·4
Sub-total hysterectomy	16	1·9
Myomectomy	2	0·2
Unilateral oöphorectomy	19	2·2
Bilateral ,,	4	0·5
Mastectomy for cancer	4	0·5
Some other abdominal operation (usually appendicectomy)	53	6·2
None	675	78·6
Not stated	56	6·5
History of wearing a pessary.		
Present	23	2·7
No history	92	10·7
Not stated	744	86·6
History of puerperal lacerations.		
Perineal or other parturition lacerations	177	20·6
No history	488	56·8
Not stated	194	22·6
History of pelvic infections.		
Any pelvic infection	30	3·5
No history	615	71·6
Not stated	214	24·9

It is probable that in many cases the blanks in the replies to the questions were meant to be negative answers, but as the figures stand they do not admit of any conclusions being drawn.

First Symptom.

	No.	Per cent.	
Irregular haemorrhage	457	53·2	67·5
Sudden profuse haemorrhage	98	11·4	
Bleeding after coitus	25	2·9	
Vaginal discharge	172	20·0	
Pain	64	7·5	
Disorders of micturition	10	1·2	
Symptoms due to secondaries	7	0·8	
Rectal symptoms	6	0·7	
Loss of weight	4	0·5	
No pelvic symptoms; growth discovered during examination	3	0·3	
Not stated	13	1·5	

Symptoms due to secondaries in 7 patients were due to intra-abdominal metastases in 3 patients, to metastases in the brain in 1, in the lung in 1, and in the spinal column and pelvic bones in 2.

Interval from First Symptom to First Consultation.

	No.	Per cent.
1 month and under	214	
1–2 months	101	45·5
2–3 ,	76	
3–4 ,,	66	
4–6 ,,	112	20·7
6–9 ,,	69	
9–12 ,,	57	
12–18 ,,	41	
18–24 ,,	26	25·6
24–48 ,,	21	
Over 48 months	6	
Not stated	70	8·1

These figures show that there was undue delay in consulting a doctor by more than half the patients, and in 25·6 per cent more than six months elapsed before advice was sought.

Interval from First Consultation to First Vaginal Examination.

	No.	Per cent.
Done at first consultation	610	71·0
Within 1 month	79	9·2
1–2 months later	19	
2–3 ,, ,,	14	3·8
3–4 ,, ,,	8	
4–5 ,, ,,	13	3·6
5–6 ,, ,,	10	
6–8 ,, ,,	8	
8–10 ,, ,,	2	2·1
10–12 ,, ,,	8	
Over 12 months later	5	0·6
Not stated	83	9·7

Advice and Treatment before Admission to Hospital.

	No.
No doctor consulted prior to coming to hospital	122
Referred to hospital without delay	583
,, ,, ,, but delayed going there	27
Treated symptomatically for periods up to 3 months before reference	29
Treated symptomatically for periods over 3 months before reference	57
Reassured or kept under observation	25
Not stated	16
Refused treatment	4

The figures in the last two tables show that in 80·2 per cent of patients a vaginal examination was made at, or within one month of the first consultation, and that of the 737 who consulted a doctor, 79·1 per cent were referred to hospital at once. A percentage of 7·7 was kept under symptomatic treatment for more than three months, but this figure included patients who were unsuitable for treatment, either by reason of poor general condition or the disease being too advanced. In 25 instances (3·4 per cent) the patient was not examined *per vaginam* for three or four months and then, when the examination revealed the true nature of the disease, was referred to hospital.

Symptoms on Admission to Hospital.

These are shown in order of frequency of occurrence, though most patients complained of more than one symptom.

	No.	Per cent.
Vaginal haemorrhage	777	90·5
,, discharge	610	71·0
Pain	417	48·5
Loss of weight	334	38·9
Disorders of micturition	119	13·9
Anaemia	64	7·5
Rectal symptoms	11	1·3
Symptoms due to secondaries	8	0·9
,, ,, intestinal obstruction	1	0·1

Findings on Examination.

The type and extent of the disease as found clinically was recorded in the stages defined in the League of Nations Classification (1937).

	No.	Per cent.
Stage I: The carcinoma is strictly confined to the cervix	201	23·4
Stage II: The carcinoma infiltrates the parametrium on one or both sides, but has not invaded the pelvic wall. Upper one-third of vagina infiltrated. Spread to corpus	318	37·0
Stage III: The carcinomatous infiltration of the parametrium has invaded the pelvic wall on one or both sides. Lower one-third of vagina involved. Isolatedmetastases on pelvic wall	221	23·7
Stage IV: Carcinoma involves bladder as determined cystoscopically, or a vesico-vaginal fistula is present. Rectum involved. Distant metastases present	113	13·2
Not staged for lack of data	6	0·7
Totals	859	100·0

Cytoscopy—Stage IV cases only.

	No.	Per cent.
Done and confirms invasion of bladder by growth	10	—
,, does not confirm invasion of bladder by growth	2	—
Not done	101	—
Recto-vaginal and vesico-vaginal fistulae :		
Recto-vaginal fistula present	8	—
Vesico-vaginal ,, ,,	11	—
No fistula present	76	—
Not stated	18	—
Remote metastases—outside pelvis :		
None found on clinical examination	693	81·5
,, after radiological examination	7	
Metastases present	34	4·0
Not stated	125	14·6
Sites of metastases in 34 patients—multiple in two :		
Abdominal lymph nodes and peritoneum	23	—
Liver	7	—
Skin and subcutaneous tissues	2	—
Lungs	1	—
Spine	1	—
Bones of pelvis	1	—
Brain	1	—

Wassermann Reaction.

The Wassermann reaction was tested in 42 patients, and was found to be positive in 12, negative in 30.

General Condition by Stages.

No. in each stage.	Stage I. 201.	Stage II. 318.	Stage III. 221.	Stage IV. 113.	Not staged. 6.	Total. 859.
Good ; no weight loss	143	176	61	12	1	393
Obese, but otherwise good	6	12	7	—	1	26
Fair ; moderate weight loss (up to 2 st.)	38	89	94	35	1	257
Poor ; considerable weight loss (over 2 st.)	9	28	44	44	3	128
Emaciated	1	4	10	15	—	30
Moribund	—	—	—	6	—	6
Not stated	4	9	5	1	—	19
Per cent in good condition	74·1	59·1	30·8	10·6	33·3	48·8

Other co-existing diseases: Seven patients were found to be suffering from diabetes, 18 from cardio-vascular disease, 1 from pulmonary tuberculosis, and 6 from mental affection.

Clinical Stages.

On page 441 the cases were grouped in the four clinical stages defined in the League of Nations Classification (1937), and in the following paragraphs the methods of treatment and the results are analysed in the same groups, so that they may be comparable with similar published series of cases. In the cases of patients who were operated on, the operation findings, or in those who died soon after admission, the autopsy findings, were used to correct the staging based on clinical signs alone, with the result that 18 cases previously classified as belonging to Stages I and II were transferred to lower stages.

	Clinical stages.	Final stages.	Difference.
Stage I	201	191	— 10
„ II	318	310	— 8
„ III	221	238	+ 17
„ IV	113	115	+ 2
Not staged	6	5	— 1

Methods of Treatment and Five-year Results.

All deaths from any cause within one month of an operation, whether radical or palliative, were counted as "operation fatalities." In the case of radiotherapeutic treatment, those patients whose death appeared to have been accelerated by the effects of radiotherapy have been classified as "died from the effects of treatment," regardless of the time which had elapsed since treatment was completed.

Radical surgical methods.	No.	Operation fatalities.	Died from effects of treatment.	Survived 5 years.	Died with cancer.	Died without cancer.	Not traced.
Wertheim's hysterectomy	34	5	—	18	9	1	1
Panhysterectomy with excision of tubes and ovaries	1	—	—	—	1	—	—
Panhysterectomy	1	—	—	1	—	—	—
Vaginal hysterectomy	1	—	—	—	—	—	1
	37	5	—	19	10	1	2
Radical combined methods.							
Wertheim's hysterectomy preceded by radium	11	1	—	6	3	1	—
Wertheim's hysterectomy preceded by H.V. X-rays	1	—	—	1	—	—	—
Wertheim's hysterectomy followed by H.V. X-rays	11	—	—	5	6	—	—
Panhysterectomy with excision of tubes and ovaries preceded by radium	3	—	—	—	2	—	1
Panhysterectomy preceded by H.V. X-rays	1	—	—	1	—	—	—
Panhysterectomy followed by H.V. X-rays	9	—	—	6	3	—	—
	36	1	—	19	14	1	1

Palliative combined methods.	No.	Operation fatalities	Died from effects of treatment.	Survived 5 years.	Died with cancer.	Died without cancer.	Not traced.
Radium combined with palliative operation (colostomy)	3	—	—	—	3	—	—
Radium after abandoned radical operation	2	—	—	1	1	—	—
	5	—	—	1	4	—	—
Radiotherapy alone.							
Radium alone	313	4	7	97	185	6	14
Radium followed by H.V. X-rays	261	—	4	77	170	5	5
H.V. X-rays followed by radium	57	—	1	19	36	1	—
H.V. X-rays alone	52	—	3	2	44	1	2
Diathermy excision followed by X-rays and radium	2	—	—	—	2	—	—
Radium preceded by drainage of pyometra	12	1	—	3	8	—	—
	697	5	15	198	445	13	21
Palliative operations and not treated.							
Exploratory laparotomy alone	8	2	—	—	6	—	—
Laparotomy for intestinal obstruction	2	2	—	—	—	—	—
Refused the treatment advised	10	—	—	1	8	—	1
Not treated by surgery or radiotherapy	64	—	—	—	64	—	—
	84	4	—	1	78	—	1
Totals of all cases	859	15	15	238	551	15	25

There were 5 "operation fatalities" following application of radium; 2 of these were due to pulmonary embolism, 1 to femoral thrombosis, 1 to general peritonitis, and 1 to post-anaesthetic pneumonia. Fifteen patients were classified as "died from effects of treatment," 5 were from radio-necrosis, 2 from general peritonitis, 3 from local peritonitis, 2 from sepsis, and 1 each from pulmonary complications, femoral thrombosis and radiation sickness. In the case of one patient who refused treatment but survived five years, the diagnosis was considered to have been mistaken, as subsequent examination revealed no clinical evidence of malignancy.

Duration of Symptoms at Time of Commencing Treatment.

	No.	Survived.	Per cent.
1 month and under	80	18	
1–3 months	166	64	30·9
3–6 ,,	213	60	
6–12 ,,	158	56	
Over 12 months	134	32	30·1
Not known	24	7	—
	775	237	

The estimates of the duration of symptoms, like those given on page 440, are based on the patients' statements. It will be seen that there is no significant difference between the survival rates of those who came under treatment within the first six months of noticing symptoms and those in whose cases the delay

was longer. The former constitute 59·3 per cent of the treated patients, and the latter 37·6 per cent. This must not be taken to mean that there is no difference between the survival rates of patients in whom the disease was diagnosed and treated in the early stages and those who were in the later stages when they came under treatment.

Operation Findings.

Forty-six patients who were classified clinically as Stage I underwent laparotomy, 8 of whom were found to have metastases in the pelvic lymph nodes, which placed them in Stage III, 1 had metastases in the paraortic lymph nodes, and 1 had involvement of the bladder, so they belonged to Stage IV. Of 24 patients classified clinically as Stage II and operated on, 8 were found to have metastases in the pelvic lymph nodes and so to be in Stage III.

Five-year Follow-up Results for all Cases.

	First year.	Second year.	Third year.	Fourth year.	Fifth year.	Totals.		
Operation fatalities	15	—	—	—	—	15		
Died from effects of treatment	14	1	—	—	—	15	596	
Died with cancer	292	136	73	35	15	551		
„ without cancer	3	2	5	2	3	15		
Alive and well	..	..	..	..	150			859
„ with cancer	..	..	..	..	25	..	238	
„ state unknown	..	..	..	..	63			
Untraced	..	..	..	..	25	..	25	
Total died each year	324	139	78	37	18			
Percentage of all cases	37·7	16·2	9·1	4·3	2·1			

The 5-year survival rate for all cases was 27·7 per cent. Of the 238 survivors, 6 are known to have died of cancer in the sixth year, 1 in the seventh, 2 in the eighth, and 1 in the tenth year. Two patients who had been successfully treated by radium died of other causes in the sixth and seventh years respectively.

Analysis of Five-year Results by Methods of Treatment and Stages.

Radical surgical methods.	Stage I.	Stage II.	Stage III.	Stage IV.	Not staged.	Total.
Wertheim's hysterectomy	22	12	—	—	—	34
Known survivors	14	4	—	—	—	18
Per cent survived	63·3	33·3	—	—	—	53·0
Per cent of traced cases	66·6	33·3	—	—	—	54·5
Panhysterectomy, abdominal or vaginal	2	1	—	—	—	3
Known survivors	1	—	—	—	—	1
Per cent survived	50·0	0·0	—	—	—	33·3
Per cent of traced cases	100·0	0·0	—	—	—	50·0
Total of radical surgical methods	24	13	—	—	—	37
Known survivors	15	4	—	—	—	19
Per cent survived	62·5	30·8	—	—	—	51·4
Per cent of traced cases	68·1	30·8	—	—	—	54·3
Radical combined methods.						
Wertheim's hysterectomy preceded by radium	7	4	—	—	—	11
Survivors	5	1	—	—	—	6
Per cent survived	71·4	25·0	—	—	—	54·5
Wertheim's hysterectomy preceded or followed by H.V. X-rays	5	5	1	—	1	12
Survivors	4	1	—	—	1	6
Per cent survived	80·0	20·0	0·0	—	100·0	50·0

Radical combined methods—Cont.	Stage I.	Stage II.	Stage III.	Stage IV.	Not staged.	Total.
Panhysterectomy preceded by radium	1	2	—	—	—	3
Known survivors	—	—	—	—	—	—
Per cent survived	0·0	0·0	—	—	—	0·0
Panhysterectomy preceded or followed by H.V. X-rays	9	—	—	1	—	10
Survivors	7	—	—	—	—	7
Per cent survived	77·7	—	—	0·0	—	70·0
Total of radical combined methods	22	11	1	1	1	36
Known survivors	16	2	—	—	1	19
Per cent survived	72·7	18·2	0·0	0·0	100·0	52·8
Per cent of traced cases	72·7	20·0	0·0	0·0	100·0	54·3
Radiotherapy alone.						
Radium alone	87	134	76	16	—	313
Known survivors	45	45	7	—	—	97
Per cent survived	51·7	33·6	9·2	0·0	—	31·0
Per cent of traced cases	55·5	34·9	9·5	0·0	—	32·4
Radium followed by H.V. X-rays	43	126	75	15	2	261
Known survivors	20	37	17	2	1	77
Per cent survived	46·5	29·4	22·7	13·3	50·0	29·5
Per cent of traced cases	47·6	30·1	23·0	13·3	50·0	30·1
H.V. X-rays followed by radium	13	15	20	8	1	57
Survivors	5	6	5	2	1	19
Per cent survived	38·5	40·0	25·0	25·0	100·0	33·3
H.V. X-rays alone	3	6	23	20	—	52
Known survivors	1	—	1	—	—	2
Per cent survived	33·3	0·0	4·3	0·0	—	3·8
Per cent of traced cases	33·3	0·0	4·5	0·0	—	4·0
Total of radiotherapeutic methods	149	288	198	59	3	697
Known survivors	72	89	31	4	2	198
Per cent survived	48·3	30·9	15·7	6·8	66·6	28·4
Per cent of traced cases	50·7	31·8	16·0	7·3	66·6	29·3
Total of all cases, treated and not treated	201	318	221	113	6	859
Known survivors	104	95	31	5	3	238
Per cent survived	51·7	29·9	14·0	4·4	50·0	27·7
Per cent of traced cases	54·2	30·7	14·3	4·5	50·0	28·5

Analysis of Five-year Results by Ages in Relation to Methods of Treatment. Stages I and II only.

	Wertheim's hysterectomy with or without radiotherapy.			Radium alone.		
Age group.	Total No.	Known survivors.	Per cent.	Total No.	Known survivors.	Per cent.
25–	4	2	—	6	3	—
35–	16	10	—	40	20	—
45–	17	6	—	71	26	—
55–	17	10	—	65	25	—
65–	1	1	—	33	14	—
75–	—	—	—	6	2	—
	55	29	52·7	221	90	40·7
	$\chi^2 = 4·00$ $P < 0·50 > 0·30$			$\chi^2 = 2·43$ $P < 0·80 > 0·70$		

These figures show that there are no statistically significant variations in the survival rates of the different age-groups. Similar calculations for the two stages separately gave the same results.

Estimation of Survival after Treatment.

Dr. Stocks, to whom this question was referred, advised that unless the follow-up of cases makes it possible to assign accurately every death, either to cancer on the one hand, or to intercurrent causes on the other, the only sound method of dealing with the duration of survival is an actuarial one, which means calculating from a life-table the total months which would be lived in the period of observation by a group of people in the general population having the same sex-age distribution as the group of patients dealt with. This gives the mean number of months expected to be lived during the five years by each group. The mean number of months actually lived is then calculated and expressed as a percentage of the normal expectation for that group, making allowances for cases followed up for less than five years.

English Life Table No. 10 (1930–32) was used for ascertaining the expectation of life.

Radical surgery alone.

Wertheim's hysterectomy	34 cases	37
Panhysterectomy	3 ,,	

	Stage I.	Stage II.
Number of cases of known duration	23	13
Mean number of months lived in 5 years from onset — Maximum possible	59·48	60·00
Expected	57·76	57·91
Actual	50·35	34·31
Per cent of Expected	87·17	59·25

Radical surgery combined with radiotherapy.

Wertheim's hysterectomy	23 cases	36
Panhysterectomy	13 ,,	

	Stage I.	Stage II.
Number of cases of known duration	22	10
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00
Expected	58·46	58·74
Actual	51·41	30·10
Per cent of Expected	87·94	51·24

Radium alone.

	Stage I.	Stage II.	Stage III.
Number of cases of known duration	79	126	69
Mean number of months lived in 5 years from onset — Maximum possible	59·85	60·00	59·65
Expected	56·83	57·06	56·21
Actual	48·71	39·10	24·14
Per cent of Expected	85·71	68·52	42·95

Radium followed by X-rays.	Stage I.	Stage II.	Stage III.
Number of cases of known duration	40	122	73
Mean number of months lived in 5 years from onset — Maximum possible	60·00	59·90	59.84
Expected	57·36	57·08	56·86
Actual	45·65	37·84	32·22
Per cent of Expected	79·59	66·29	56·67

H.V. X-rays followed by radium.			
Number of cases of known duration	13	14	20
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00
Expected	58·29	57·87	58.12
Actual	36·85	42·14	30·25
Per cent of Expected	63·22	72·82	52·05

X-rays alone.			
Number of cases of known duration	3	5	22
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	60·00
Expected	57·84	54·64	56·04
Actual	39·33	20·80	19·86
Per cent of Expected	68·00	38·07	35·44

Not treated by surgery or radiotherapy.	All stages.
Number of cases of known duration	64
Mean number of months lived in 5 years from onset — Maximum possible	60·00
Expected	55·44
Actual	14·36
Per cent of Expected	25·90

These figures show that in Stage I hysterectomy gave the best results, which slightly improved if operation was preceded by radium treatment; the results with radium alone were almost as good as those of operation, and were not improved by courses of X-rays in addition. In Stage II both the 5-year survival rate and the expectation of life were better after radium treatment than after surgery, and in one small group a preliminary course of X-rays slightly improved the results. In Stage III almost all patients were treated by radium, and it was found that a course of X-rays, either before or after the radium treatment, gave improved 5-year survival rate and expectation of life.

Biopsy.

	No.	Per cent.
Biopsy done before or at commencement of treatment	629	79·6
,, ,, during radiation treatment	55	
Not done	175	20·4

Pathological Report.

	No.	Per cent.
No histological examination before or after death	141	16·4
Histological examination done before or after death	718	83·6
Result doubtful	3	
The specimen examined was non-malignant	13	
Squamous cell carcinoma, keratinizing	541	
,, ,, ,, non-keratinizing	29	
,, ,, ,, undifferentiated	37	
Carcinoma, type unspecified	23	
Spheroidal cell carcinoma	10	
Adenocarcinoma	44	
,, colloid	1	
,, papillary	10	
Transitional cell carcinoma	2	
Basal cell carcinoma	3	
Chorion-carcinoma	1	
Spindle cell sarcoma	1	

Basis of diagnosis in 859 *cases.*

Clinically malignant :

Confirmed by histological examination and/or autopsy	781	90·9
,, by appearance of metastases or recurrence	11	1·3
Diagnosis based on clinical evidence only	66	7·7

Clinically benign :

Proved malignant by appearance of recurrence	1	0·1

Carcinoma of Cervix following Supravaginal Hysterectomy.

There were 16 patients (1·9 per cent) who had undergone supravaginal hysterectomy, usually for the treatment of fibroids 1–18 years previously, mean interval 9·5 years. The mean age was 53 years. There were 3 nulliparae, one 1-para, and the remainder were multiparae. Five patients were classified as in Stage I, 5 in Stage II, 5 in Stage III, and one in Stage IV. Two patients were treated by radical surgery with one operation fatality and one survival; 5 by radium alone, of whom 2 have survived 5 years, but one of these died of cancer in the eighth year; 9 by radium with X-rays, of whom 3 have survived 5 years. The diagnosis of carcinoma was confirmed by biopsy in 15 patients, in 12 of whom the specimen was reported as keratinizing squamous cell carcinoma, in one as undifferentiated squamous cell carcinoma, in one as columnar cell carcinoma, and in one as papillary adenocarcinoma, the latter being the patient who died of recurrence in the eighth year.

Other Primary Growths.

Previous primary tumours—3 patients.

1. Age 58. Simple mastectomy for histologically confirmed spheroidal cell carcinoma of breast, two years previously. Admitted with metastases in vertebrae and a Stage I growth in the cervix which proved to be a papillary adenocarcinoma. Treated by X-rays. Known to be alive at the end of the fifth year.

2. Age 64. Had had partial cystectomy for cancer of the bladder (histology not recorded) 5 years previously; no recurrence. Admitted with Stage II prickle-cell carcinoma of cervix. Radium treatment. Died in first year.

3. Age 69. Had oöphorectomy for papillary adenocarcinoma 4 years previously; no recurrence. Admitted with a Stage II carcinoma of cervix of same histology. Radium and H.V. X-ray treatment. Died in the fourth year.

Simultaneous primary growths—4 patients.

4. Age 50. Stage II carcinoma of cervix and rodent ulcer of forehead. No histology. H.V. X-rays to cervix; refused to continue treatment. Died in second year.

5. Age 58. Stage II squamous cell carcinoma of cervix. Treated by radium and H.V. X-rays; no recurrence. Simultaneous spheroidal cell carcinoma of breast, Stage III. Treated by H.V. X-rays and radium. Died in third year from metastases of the breast growth.

6. Age 65. Carcinoma of cervix, Stage IV, no histology. Treated by radium. Also rodent ulcer of right eyelid, which recurred after excision and was histologically carcinoma. Metastases in cervical nodes led to death in third year.

7. Age 65. Carcinoma of cervix, Stage III. No treatment. A separate cauliflower growth found in rectum at autopsy. No histology of either growth.

Subsequent primary growths—5 patients.

8. Age 55. Squamous cell carcinoma of cervix, Stage II. Radium and H.V. X-ray treatment. Local recurrence in third year. A carcinoma of the right breast was found in the third year and treated by simple mastectomy. Alive with cancer at the end of the fifth year.

9. Age 50. Carcinoma of cervix, Stage II; histologically not malignant. Treated by radium and H.V. X-rays. No recurrence. Carcinoma of breast in the seventh year (no histology), treated by radon needling. Alive with cancer at the end of the seventh year.

10. Age 46. Squamous cell carcinoma of cervix, Stage I. Treated by radium, no recurrence. Died in the third year from an adenocarcinoma of the bronchus.

11. Age 58. Squamous cell carcinoma of cervix, Stage II. Treated by radium and H.V. X-rays. Generalized recurrence in the third year. Clinically and radiologically there was also a carcinoma of oesophagus; no biopsy. Died in the third year.

12. Age 64. Squamous cell carcinoma of cervix, Stage II. Treated by radium and H.V. X-rays. Carcinoma of breast in the seventh year, spheroidal cell carcinoma, with involvement of nodes. Died two and a half years later from recurrence of the growth in the cervix.

Cause of Death in 596 Patients.

	No.
Cachexia	428
Uraemia	34
Pulmonary complications	32
Haemorrhage	17
Peritonitis	14
Cardio-vascular disease	13
Sepsis	11
Embolism (pulmonary 5, cerebral 1, local 1)	7
Intestinal obstruction	6
Cerebral	4
Surgical shock	3
Intercurrent disease or unknown cause	27

Autopsy.

	No.	Per cent.
No autopsy	514	86·3
Autopsy done	82	13·8

Relevant autopsy findings (multiple in some cases).	No.
Local growth only	11
Extension to neighbouring parts	47
Metastases in pelvic lymph nodes	12
,, abdominal lymph nodes	22
,, liver and abdominal organs	20
,, lungs and pleurae	8
,, brain	3
,, skeletal system	6
Pulmonary complications	19
Abdominal ,,	16
No growth found (operated cases)	4

RECURRENT CASES.

There were 96 recurrent cases, of which 13 followed Wertheim's hysterectomy, and 83 treatment by radium, with or without H.V. X-rays. The relation of the type of recurrence to the method of treatment of the primary with the mean intervals of freedom are shown below.

Following Wertheim's hysterectomy—13 cases.	No.	Mean interval of freedom in months.
Local recurrence only	7	14·7
Recurrence deep in pelvis	3	70·0
Both local and pelvic	2	6·0
Distant metastases	2	8·0

In one patient there were both distant and pelvic metastases. The shortest interval was 3 months and the longest 144 months.

Following radium treatment—83 cases.	No.	Mean interval of freedom in months.
Local recurrence	13	18·0
Recurrence deep in pelvis	25	25·4
Both local and pelvic	38	24·4
Distant metastases	13	23·2

In 6 patients there were both distant and pelvic metastases. The shortest interval was 2 months, and the longest 144 months.

Six of the 13 patients with recurrences following hysterectomy received further treatment: with radium, one patient; radium and H.V. X-rays, 2; X-rays alone, 3; colostomy was performed for the relief of intestinal obstruction in 2, and 5 were not treated. All 13 patients are dead.

Thirty of the 83 patients with recurrences following radium treatment received further treatment. Three with radium alone for local recurrence; 4 with radium and H.V. X-rays; 20 with H.V. X-rays alone; 2 with H.V. X-rays following colostomy; one by colostomy alone, and 53 were not treated. One patient is still alive, 81 are dead and 1 is untraced.

SUMMARY.

1. A statistical analysis of 955 cases of cancer of the cervix uteri. 859 of these were primary cases.

2. 4·3 per cent of the patients were single women and 95·3 per cent were married or widowed. The mean age of the single women was 51·5 years, and that of the married and widowed 54·7.

3. Analysis of the ratios of married and widowed to single women by age groups led to the conclusion that liability to cancer of the cervix uteri was greater at every age amongst married and widowed than amongst single women, and that between the ages of 45 and 65 it was about seven times as great.

4. There were 91 nulliparous patients in all, and amongst 787 married and widowed patients of known parity there were 71 nulliparae against 135 expected (calculated from the percentages of childless married and widowed women in each age group registered as dying from all causes in England and Wales in 1939), indicating that parous married women were more liable to this form of cancer than nulliparous women.

5. Irregular haemorrhage was the first symptom in 67·5 per cent of the patients, vaginal discharge in 20 per cent.

6. 45·5 per cent of the patients consulted a doctor within three months of noticing the first symptoms, 20·7 per cent within the next three months, and in 25·6 per cent the symptoms were of more than six months' duration before advice was sought. In 27 cases the delay was more than two years.

7. In 71 per cent a vaginal examination was made at the first consultation, and in a further 9·2 per cent it was done within one month of the first consultation. In only 10·1 per cent was the examination delayed longer than one month.

8. 79·1 per cent of the patients who consulted a doctor were referred to hospital forthwith, 3·9 per cent were treated symptomatically for periods up to three months before reference, and 7·7 per cent were kept under treatment for more than three months, but this figure included patients who were unsuitable for active treatment by reason of poor general condition or the disease being

too advanced. In 3·4 per cent the patient was told that there was nothing serious the matter until, some months later, examination revealed the true nature of the disease.

9. On admission to hospital the cases were classified according to the League of Nations Classification (1937) into four stages, Stage I 23·4 per cent, Stage II 37·0, Stage III 25·7, Stage IV 13·2, and cases not staged for lack of data 0·7 per cent,

10. Thirty-four patients in Stages I and II were treated by Wertheim's hysterectomy with an operation mortality of 14·7 per cent, and a 5-year survival rate of 66·6 per cent of traced cases in Stage I and 33·3 per cent in Stage II. Estimated actuarially, the 5-year expectation of life for radical operations was 87·17 and 59·25 per cent of normal expectation respectively.

11. Thirty-six patients were treated by Wertheim's hysterectomy or pan-hysterectomy preceded or followed by radiotherapy, with an operative mortality of 2·8 per cent. The 5-year survival rate averaged 72·7 per cent of traced cases in Stage I and 20 per cent in Stage II. Estimated actuarially the 5-year expectation of life was 87·9 per cent of normal expectation in Stage I and 51·2 per cent in Stage II.

12. Six hundred and ninety-seven patients were treated by radiotherapy alone with 5 operation fatalities following radium applications, and 15 in whom death was due to the effects of treatment—radio-necrosis, peritonitis, pulmonary complications, etc.—making 2·9 per cent.

Three hundred and thirteen patients were treated by radium alone with a 5-year survival rate of 55·5 per cent of traced cases in Stage I, and 34·9 per cent of traced cases in Stage II. Actuarially estimated, the 5-year expectation of life was 85·7 per cent of normal expectation in Stage I and 68·5 per cent in Stage II. For all methods of radiotherapy the average 5-year survival rates were 50·7 per cent of traced cases in Stage I, 31·8 per cent in Stage II, 16 per cent in Stage III, and 7·3 per cent in Stage IV.

13. Sixty-four patients were not treated either by surgery or by radiotherapy; all are dead after an average duration of life of 14·36 months, which is 25·90 per cent of the normal 5-year expectation of life.

14. Biopsy was done in 79·6 per cent of the patients and there were histological reports for 83·6 per cent.

15. In 16 patients the growth arose in the stump of the cervix remaining after supravaginal hysterectomy, performed on an average 9·5 years previously. Two patients were treated by radical surgery of whom 1 survived, and 14 by radiotherapy of whom 5 survived five years.

16. There were 13 patients with recurrent growths following Wertheim's hysterectomy, and 83 following radiotherapy.

PART II: CANCER OF THE CORPUS UTERI.

There were 288 primary and 33 recurrent cases.

Primary Cases.

Civil State.

	No.		Per cent.
Single	66		22·9
Married	151	222	77·1
Widowed	71		

Lane-Claypon and McCullagh (1927) give the civil state of 207 women suffering from cancer of the corpus uteri as 22·7 per cent single and 77·3 per cent married or widowed; the percentage of single women amongst those suffering from cancer of the cervix was 3·2 and the difference of 19·5±2·01 was statistically significant. Our figures of 22·9 per cent and 4·3 per cent (Part I) also show a statistically significant difference of 18·6±2·57.

Age Distribution.

Age groups.	Single.	Married and widowed.	Total.
25–34	—	5	5
35–39	2	4	6
40–44	4	8	12
45–49	4	25	29
50–54	15	35	50
55–59	16	41	57
60–64	10	44	54
65–69	5	28	33
70–74	6	24	30
75–	4	8	12
Mean age	58·3±1·17	58·6±0·68	58·5±0·59
Standard deviation	9·5±0·83	10·2±0·48	10±0·42

The higher mean age as compared with that of patients with cancer of the cervix is in accord with general experience. The ratios of married and widowed to single women by age groups compared with the corresponding ratios for cancer of the cervix, with Dr. Stocks' comments, have already been given in Part I, page 434.

Heredity.

	No.	Per cent.
Family history of cancer	35	12·2±1·9
,, ,, ,, in more than one relative	9	3·1
,, ,, ,, of the uterus	3	1·0
No family history of cancer	154	53·5±2·9
Not stated	99	34·3±2·8

The family histories of the 35 patients who gave a history of cancer in the family were examined in more detail, only parents and siblings being included.

	No.	(1) Per cent of all patients of known family history.	(2) Per cent of all patients.
Father suffered from cancer of any other site	9	4·8±1·55	3·1±1·02
Mother suffered from cancer of any other site	11	7·4±1·9	4·9±1·27
Mother suffered from cancer of uterus	3		
Brother(s) suffered from cancer of any other site	7	3·7±1·37	2·4±0·9
Sister(s) suffered from cancer of any other site	14	7·4±1·9	4·9±1·27
Sister(s) suffered from cancer of uterus	—	—	—

The percentages give the high and low values with the 99 patients for whom no family history was obtained excluded from Column 1 and included in Column 2. There was only one instance of both parents having died of cancer among the 189 patients whose family history was known, which is about the same proportion as the expected frequency for all regions of the body (Stocks and Karn, 1933). Although the percentages are slightly higher than those for the cervix uteri, they are not so high as to suggest that heredity is a factor in the aetiology.

Relevant Past History.

Menstrual cycle.

Questions were put as to the length of the cycle, duration and amount of flow and presence of any dysmenorrhoea. The questions were answered by 74·0 per cent of the patients. The interval was normal in 67·7 per cent, duration was normal in 43·7 per cent, prolonged in 17·4 per cent. The amount lost was recorded as scanty in 7·3 per cent, normal in 46·2 per cent, and excessive in 17 per cent. Dysmenorrhoea, either premarital only, or at all times was reported by 6·2 per cent. The percentages of deviation from normal are greater than in the case of the cervix uteri, and greater than those recorded for a control series of 509 women (Lane-Claypon, 1926), but the percentages are invalidated by 26 per cent of " not stated."

The menopause.

The relationship of the onset of the disease to the menopause and the age at which the latter occurred were recorded separately for the single and for the married and widowed.

	Single.	Married and widowed.	Total.	
			No.	Per cent.
Menopause not yet reached . . .	12	43	55	19·1
,, was artificially induced . .	1	3	4	1·4
Patient was past the menopause . .	45	155	200	69·4
Not stated	8	21	29	10·1
Age at menopause : Under 40 . .	—	6	6	2·9
40– . .	1	8	9	4·4
44– . .	10	25	35	17·2
48– . .	21	71	92	45·1
52– . .	13	42	55	27·0
56 and over .	1	6	7	3·4
Interval between the menopause and onset of symptoms : 0–5 years . . .	15	40	55	27·0
5–10 ,, . . .	13	36	49	24·0
Over 10 ,, . . .	18	82	100	49·0
Total number who were past the menopause	46	158	204	100·0

When compared with the corresponding figures for the cervix uteri it will be seen that 11·2 per cent more of those with cancer of the corpus uteri were past the menopause, owing to the later age of onset of the disease. Three patients had had an artificial menopause induced by means of radium for the treatment

of menorrhagia and one by oöphorectomy. Lane-Claypon (1926) found that 29 of 509 control patients (5·7 per cent) had had non-cancerous conditions (Part I, p. 435).

Children and miscarriages.	Single.	Married and widowed.	Total.
No children	55	44	99
One child	—	59	59
Two children	—	31	31
Three ,,	—	29	29
Four ,,	—	16	16
Five or more children	—	23	23
Not stated	11	20	31
No miscarriages	53	141	194
One miscarriage	—	34	34
Two miscarriages	—	7	7
Three or more miscarriages	—	7	7
Not stated	13	33	46

The 99 nulliparous patients were divided into those who were totally barren and those who had had one or more miscarriages :

	Single.	Married and widowed.	Total.
No children, no miscarriages	55	37	92
,, but one or more miscarriages	—	7	7

Of 257 women of known parity (288, less 31 unknown), 99 or 34·6 per cent were nulliparous, and 92 or 31·9 per cent were completely sterile. Lane-Claypon (1927) found from data in the literature that 29 per cent of 389 patients with cancer of the corpus were nulliparous. Dr. Stocks' comments on the figures have already been given in Part I, page 438.

Interval between last pregnancy and first symptom of cancer in 257 patients of known parity.

No children or miscarriages	92
Pregnant at the time	—
Less than one year ago	1
1–5 years ago	3
5–10 ,,	6
10–15 ,,	14
15–20 ,,	24
20–25 ,,	15
25–30 ,,	27
30–35 ,,	20
35–40 ,,	21
Over 40 years ago	15
Not stated	19

The maximum incidence is 15–30 years after the birth of the last child, which is five years later than in the case of cancer of the cervix.

	No.	Per cent.
History of puerperal lacerations.		
Any puerperal lacerations	35	12·2
No history	178	61·8
Not stated	75	26·0
History of previous operations.		
Previous dilatation and curetting	17	5·9
Cervix operation	9	3·1
Myomectomy	1	0·3
Unilateral oöphorectomy	3	1·0
Bilateral ,,	3	1·0
Previous mastectomy for cancer	1	0·3
No history	195	67·7
Not stated	59	20·5
History of pelvic infections.		
Any pelvic infection	6	2·1
No history	174	60·4
Not stated	108	37·5
History of previous vaginal discharge.		
Present	54	18·8
Not present	140	48·6
Not stated	94	32·6
History of previous fibromyoma.		
Present	15	5·2
Not present	149	51·7
Not stated	124	43·1

First Symptom.

	No.	Per cent.	
Irregular haemorrhage	149	51·7	63·2
Sudden profuse haemorrhage	33	11·5	
Discharge	63	21·9	
Pain	20	6·9	
Disorders of micturition	6	2·1	
Abdominal tumour or swelling	5	1·7	
Loss of weight	3	1·0	
Rectal symptoms	1	0·3	
Symptoms due to secondaries	1	0·3	
No pelvic symptoms, growth discovered during examination	1	0·3	
Not stated	6	2·1	

In one patient the first symptom was haemoptysis; exploration revealed an extensive tumour diagnosed as a chorion-carcinoma following hydatiform mole five months before; the haemoptysis was probably due to a metastasis in the lung, but the patient was lost sight of.

Interval from First Symptom to First Consultation.

	No.	Per cent.
One month and under	55	
1–2 months	24	33·7
2–3 „	18	
3–4 „	9	
4–6 „	32	14·2
6–9 „	28	
9–12 „	28	
12–18 „	24	
18–24 „	17	39·6
24–48 „	6	
Over 48 months	11	
Not stated	36	12·5

These figures show that two-thirds of the patients delayed more than three months, and nearly half of them more than six months before consulting a doctor

Interval from First Consultation to First Vaginal Examination.

	No.	Per cent.
Done at first consultation	221	76·7
Within one month	20	6·9
1–2 months later	2	
2–3 „ „	5	2·4
3–4 „ „	1	
4–5 „ „	1	1·0
5–6 „ „	1	
6–8 „ „	—	
8–10 „ „	1	0·3
10–12 „ „	1	0·3
Over 12 months later	1	0·3
Not stated	34	11·8

Advice and Treatment before Admission to Hospital.

	No.
No doctor consulted prior to coming to hospital	29
Referred to hospital without delay	191
„ „ but delayed going there	11
Treated symptomatically for periods up to 3 months before reference	10
„ „ „ over 3 months before reference	17
Reassured or kept under observation	6
Not stated	24

The figures in the above two paragraphs show that in 83·6 per cent of the patients a vaginal examination was made at, or within one month of the first consultation, and that of the 259 patients who consulted a doctor 73·7 per cent were referred to hospital at once. 6·6 per cent were kept under symptomatic treatment for more than three months, but this figure includes patients who were unsuitable for treatment, either by reason of poor general condition or the disease being too advanced. In six instances (2·3 per cent) the patient was told that the symptoms were due to the menopause and that she need not worry about them.

Symptoms on Admission to Hospital.

These are shown in order of frequency of occurrence, though most patients complained of more than one symptom.

	No.	Per cent.
Haemorrhage	243	84·4
Discharge	177	61·5
Pain	112	38·9
Abdominal tumour or swelling	67	23·3
Loss of weight	40	13·9
Disorders of micturition	36	12·5
Anaemia	11	3·8
Rectal symptoms	1	0·3
Symptoms due to secondaries	3	1·0

Findings on Examination.

Size of uterus.	No.	Per cent.
Enlarged	185	64·2
Not enlarged	66	22·9
Not stated	37	12·8
Mobility of uterus.		
Freely mobile	194	67·4
Mobility limited	24	8·3
Fixed	42	14·6
Not stated	28	9·7
Involvement of cervix.		
Involved	22	7·6
Not involved	233	80·9
Not stated	33	11·5
Fibromyoma present simultaneously.		
Present	30	10·4
Not present	216	75·0
Not stated	42	14·6

Type of fibromyoma.	No.
Not specified	15
Subserous	4
Submucous	2
Massive	9

Norris and Vogt (1924) found that 20·8 per cent of cases of fundus carcinoma were associated with myoma, and that the symptoms of myoma usually obscured those of carcinoma. The relationship between sarcoma and myoma in this series of cases is dealt with on page 469.

Extension to Neighbouring Organs.

	No.	Per cent.
Vagina.		
Involved by extension	12	4·2
,, isolated metastatic nodules	6	2·1
Not involved	242	84·0
Not stated	28	9·7
Broad ligament.		
Involved	39	13·5
Not involved	208	72·2
Not stated	41	14·2
Bladder.		
Involved	6	2·1
Involvement confirmed by cystoscopy	2	
Not involved	239	83·0
Not stated	43	14·9
Rectum.		
Involved	11	3·8
Not involved	247	85·8
Not stated	30	10·4

Local Metastases.

	No.	Per cent.
Pelvic lymph nodes.		
Pelvic masses present	44	15·3
,, ,, not present	219	76·0
Not stated	25	8·7
Inguinal lymph nodes.		
Involved	4	1·4
Not involved	221	76·7
Not stated	63	21·9

Remote Metastases.

	No.	Per cent.
None found on clinical examination	243	
,, ,, after radiological examination	1	84·7
Metastases present	20	6·9
Not stated	24	8·3

Sites of Metastases in 20 Patients (multiple in 2).

	No.
Abdominal lymph nodes and peritoneum	11
Liver and abdominal organs	6
Lungs and pleurae	2
Bones of pelvis	1
Skin and subcutaneous tissues	1
Lymph nodes other than regional	1

Wassermann Reaction.

The Wassermann reaction was tested in 11 patients and was found to be negative in all.

General Condition. Other Co-existing Diseases.

	No.	Per cent.
Good; no weight loss	130	54·9
Obese; but otherwise good	28	
Fair; moderate weight loss (up to 2 st.)	62	21·5
Poor; considerable weight loss (over 2 st.)	37	12·8
Emaciated	5	1·7
Moribund	5	1·7
Not stated	21	7·3

Four patients were found to be suffering from diabetes, and 10 from cardio-vascular disease.

Clinical Stages.

The cases were arranged in five groups according to the clinical findings given on pages 459, and 460.

	No.	Per cent.
Group I: Disease limited to corpus uteri	170	59·0
,, II: ,, has spread into parametrial tissues, tubes or cervix	37	12·8
,, III: ,, Disease had involved regional nodes, vagina, bladder or rectum	45	15·6
,, IV: ,, Remote metastases outside pelvis present	21	7·3
Not grouped for lack of data	15	5·2

In the cases of patients who underwent operation, the operation and pathological findings, where available, were used for correcting the groups into stages. Patients not operated on were placed in the appropriate stage according to the

clinical evidence of extension of the growth and the presence of metastases. The final staging is shown below :

	No.	Per cent.
Stage I : Disease limited to corpus uteri.		
Confined to corpus uteri clinically	128	
,, endometrium histologically	20	
Invading myometrium	41	
	189	65·6
Stage II : Disease has spread outside corpus uteri.		
Growth has involved the cervix uteri	10	
,, ,, peritoneum covering the uterus	6	
,, ,, parametrial tissues	20	
,, ,, tubes or ovaries	8	
	44	15·3
Stage III : Disease has involved regional lymph nodes or adjacent organs.		
Regional lymph nodes involved	9	
Vagina involved	14	
Bladder or rectum involved	5	
	28	9·7
Stage IV : Remote metastases present.		
Remote metastases outside the pelvis present	27	9·4

The differences between the clinical and final staging will be seen from the following table, which shows that more patients are still in Stages I and II than appear to be so on the clinical findings.

	Clinical stages.	Final stages.	Difference.
Stage I	170	189	+19
,, II	37	44	+7
,, III	45	28	−17
,, IV	21	27	+6
Not staged	15	—	−15

Methods of Treatment and 5-year Results.

All deaths from any cause within one month of an operation, whether radical or palliative, were counted as "operation fatalities." In the case of radiotherapeutic treatment those patients whose death appeared to have been accelerated by the effects of radiotherapy have been classified as "died from effects of treatment," regardless of the time which had elapsed since treatment was completed.

Surgical and combined methods.	No.	Operation fatalities.	Died from effects of treatment.	Survived 5 years.	Died with cancer.	Died without cancer.	Not traced.
Panhysterectomy with excision of tubes and ovaries	74	4	—	42	17	1	10
Panhysterectomy with excision of tubes and ovaries preceded by radium or by radium and X-rays	12	1	—	9	2	—	—
Panhysterectomy with excision of tubes and ovaries followed by H.V. X-rays	22	—	—	12	6	1	3
Panhysterectomy	22	5	—	8	7	1	1
Panhysterectomy preceded by radium or by radium and H.V. X-rays	5	—	—	3	1	—	1
Panhysterectomy followed by H.V. X-rays	9	—	—	7	2	—	—
Supravaginal hysterectomy	14	—	—	5	7	—	2
Supravaginal hysterectomy followed by H.V. X-rays	7	—	—	5	2	—	—
Supravaginal hysterecomy followed by radium and H.V. X-rays	1	—	—	—	1	—	—
Vaginal hysterectomy	2	—	—	2	—	—	—
Exploratory laparotomy	9	1	—	—	7	—	1
	177	11	—	93	52	3	18
Radiotherapy alone.							
Radium alone	50	2	1	22	20	5	—
Radium with H.V. X-rays	24	—	2	10	12	—	—
H.V. X-rays alone	5	—	—	2	3	—	—
H.V. X-rays following exploratory laparotomy	7	—	—	—	7	—	—
	86	2	3	34	42	5	—
Not treated by surgery or radiotherapy.	25	—	—	—	25	—	—
Total of all cases	288	13	3	127	119	8	18

There were 11 operation fatalities in 177 cases, or 6·2 per cent. There were 2 fatalities due to perforation of the infiltrated uterine wall, and one death from a pelvic abscess following radium treatment and 2 deaths from pelvic peritonitis following radium and X-ray treatment.

Analysis of Methods of Treatment by Stages.

	Stage I.	Stage II.	Stage III.	Stage IV.	Total.	Per cent. of total.
Number in each stage	189.	44.	28.	27.	288.	100·0.
Radical surgical methods	86	16	8	2	112	38·9
,, combined ,,	41	11	3	1	56	19·4
Exploratory laparotomy alone or with radiotherapy	1	6	2	7	16	5·6
Radiotherapeutic methods	58	9	9	3	79	27·4
Not treated by surgery or radiotherapy	3	2	6	14	25	8·7
Percentage treated by radical surgical or combined methods	67·2	61·4	39·3	11·1	58·3	—

Duration of Symptoms at Time of Commencing Treatment.

	No.	Survived.	Per cent.
1 month and under	10	6	
1–3 months	45	19	49·5
3–6 ,,	44	24	
6–12 ,,	72	33	
Over 12 months	84	43	48·7
Not known	8	2	
Total	263	127	

The estimates of the duration of symptoms, like those given on page 458, are based on the patients' statements. It will be seen that there is no significant difference between the survival rates of those who came under treatment within the first six months and those in whose cases the delay was longer. The former constitute 37·6 per cent of the treated patients and the latter 59·3 per cent.

Five-year Follow-up Results for all Cases.

	First year.	Second year.	Third year.	Fourth year.	Fifth year.	Totals.		
Operation fatalities	12	1	—	—	—	13		
Died from effects of treatment	3	—	—	—	—	3	143	
„ with cancer	73	30	4	7	4	118		
„ without cancer	1	3	1	1	3	9		
Alive and well	—	—	—	—	82			288
„ with cancer	—	—	—	—	9		127	
„ state unknown	—	—	—	—	36			
Untraced	—	—	—	—	18		18	
Total died each year	89	34	5	8	7			
Percentage of all cases	30·9	11·8	1·7	2·8	2·4			

The five-year survival rate for all cases was 44·1 per cent. Two of the 127 survivors are known to have died of cancer early in the sixth and ninth year respectively; six patients died of other causes without evidence of recurrence, 3 in the sixth year, 2 in the seventh, and 1 in the eighth year.

Analysis of 5-year Results by Methods of Treatment and Stages.

Surgical or combined methods.

	Stage I.	Stage II.	Stage III.	Stage IV.	Total.
Panhysterectomy with excision of tubes and ovaries	57	11	6	—	74
Known survivors	36	4	2	—	42
Per cent survived	63·2	36·4	33·3	—	56·8
Per cent of traced cases	73·5	44·4	33·3	—	65·6
Panhysterectomy with excision of tubes and ovaries preceded by radiotherapy	8	3	1	—	12
Known survivors	6	2	1	—	9
Per cent survived	75·0	66·6	100·0	—	75·0
Panhysterectomy with excision of tubes and ovaries followed by H.V. X-rays	15	5	2	—	22
Known survivors	8	3	1	—	12
Per cent survived	53·3	60·0	50·0	—	54·5
Per cent of traced cases	57·2	100·0	50·0	—	63·2
Panhysterectomy alone	15	3	2	2	22
Known survivors	7	1	—	—	8
Per cent survived	46·6	33·3	0·0	0·0	36·6
Panhysterectomy preceded by radiotherapy	4	1	—	—	5
Known survivors	3	—	—	—	3
Per cent survived	75·0	0·0	—	—	60·0
Per cent of traced cases	100·0	0·0	—	—	75·0
Panhysterectomy followed by H.V. X-rays	9	—	—	—	9
Known survivors	7	—	—	—	7
Per cent survived	77·7	—	—	—	77·7
Supravaginal hysterectomy alone	12	2	—	—	14
Known survivors	3	2	—	—	5
Per cent survived	25·0	100·0	—	—	35·7
Per cent of traced cases	30·0	100·0	—	—	41·7

Surgical or combined methods—cont.	Stage I.	Stage II.	Stage III.	Stage IV.	Total.
Supravaginal hysterectomy followed by H.V. X-rays	5	2	—	1	8
Known survivors	3	2	—	—	5
Per cent survived	60·0	100·0	—	0·0	62·5
Total of radical surgical and combined methods	127	27	11	3	168
Known survivors	75	14	4	—	93
Per cent survived	59·1	51·9	36·4	0·0	55·4
Per cent of traced cases	65·2	60·9	36·4	0·0	61·6
Radiotherapy alone.					
Radium alone	40	5	3	2	50
Known survivors	22	—	—	—	22
Per cent survived	55·0	0·0	0·0	0·0	44·0
Combinations of radium and X-rays	18	3	3	—	24
Known survivors	8	1	1	—	10
Per cent survived	44·4	33·3	33·3	—	41·7
H.V. X-rays alone	—	1	3	1	5
Known survivors	—	—	2	—	2
Per cent survived	—	0·0	66·6	0·0	40·0
Total of radiotherapeutic methods	58	9	9	3	79
Known survivors	30	1	3	—	34
Per cent survived	51·7	11·1	33·3	0·0	43·0
Total of all cases treated and not treated.					
Total number of cases	189	44	28	27	288
Known survivors	105	15	7	—	127
Per cent survived	55·5	34·1	25·0	0·0	44·1
Per cent of traced cases	59·3	38·5	25·0	0·0	47·0

Analysis of 5-year Results by Ages in Relation to Methods of Treatment in Stage 1 Cases only.

Age group.	Panhysterectomy with excision of tubes and ovaries, with or without radiotherapy.			Radium alone.		
	Total No.	Known survivors.	Per cent.	Total No.	Known survivors.	Per cent.
25–	1	1	—	—	—	—
35–	4	4	—	—	—	—
45–	32	22	—	4	4	—
55–	33	18	—	20	13	—
65–	10	5	—	13	4	—
75–	—	—	—	3	1	—
	80	50	62·5	40	22	55·0
	$\chi^2 = 5{\cdot}08$ $P < 0{\cdot}30 > 0{\cdot}20$			$\chi^2 = 7{\cdot}72$ $P < 0{\cdot}20 > 0{\cdot}10$		

In both the above examples the figures for survival by age groups are not statistically significant, as the value of P shows that this distribution might occur by chance 1 in 5 times and 1 in 10 times respectively.

Estimation of Survival after Treatment.

	Stage I.	Stage II.	Stage III.
Panhysterectomy with excision of tubes and ovaries—74 cases			
Number of cases of known duration	53	8	5
Mean number of months lived in 5 years from onset: Maximum possible	58·64	58·50	60·00
Mean number of months lived in 5 years from onset: Expected	56·09	55·47	56·27
Mean number of months lived in 5 years from onset: Actual	49·96	41·37	30·20
Per cent of Expected	89·07	74·58	53·67
Panhysterectomy with excision of tubes and ovaries preceded by radiotherapy —12 cases.			
Number of cases of known duration	8	3	1
Mean number of months lived in 5 years from onset: Maximum possible	60·00	60·00	—
Mean number of months lived in 5 years from onset: Expected	57·67	56·23	—
Mean number of months lived in 5 years from onset: Actual	49·50	60·00	—
Per cent of Expected	85·83	106·70	—
Panhysterectomy with excision of tubes and ovaries followed by radiotherapy—22 cases.			
Number of cases of known duration	15	4	2
Mean number of months lived in 5 years from onset: Maximum possible	59·20	57·00	—
Mean number of months lived in 5 years from onset: Expected	56·76	55·11	—
Mean number of months lived in 5 years from onset: Actual	45·07	57·00	—
Per cent of Expected	79·40	103·43	—
Panhysterectomy alone—22 cases.			
Number of cases of known duration	15	3	2
Mean number of months lived in 5 years from onset: Maximum possible	60·00	60·00	—
Mean number of months lived in 5 years from onset: Expected	57·46	58·16	—
Mean number of months lived in 5 years from onset: Actual	46·20	23·66	—
Per cent of Expected	80·40	40·68	—
Panhysterectomy alone preceded by radiotherapy—5 cases.			
Number of cases of known duration	3	2	—
Mean number of months lived in 5 years from onset: Maximum possible	60·00	—	—
Mean number of months lived in 5 years from onset: Expected	56·43	—	—
Mean number of months lived in 5 years from onset: Actual	60·00	—	—
Per cent of Expected	106·43	—	—

	Stage I.	Stage II.	Stage III.
Panhysterectomy alone followed by radiotherapy—9 cases.			
Number of cases of known duration	9	—	—
Mean number of months lived in 5 years from onset — Maximum possible	60·00	—	—
Mean number of months lived in 5 years from onset — Expected	58·56	—	—
Mean number of months lived in 5 years from onset — Actual	60·00	—	—
Per cent of Expected	102·46	—	—
Supravaginal hysterectomy alone—14 cases.			
Number of cases of known duration	10	2	—
Mean number of months lived in 5 years from onset — Maximum possible	56·40	—	—
Mean number of months lived in 5 years from onset — Expected	53·96	—	—
Mean number of months lived in 5 years from onset — Actual	38·10	—	—
Per cent of Expected	79·87	—	—
Supravaginal hysterectomy followed by radiotherapy—8 cases.			
Number of cases of known duration	4	2	—
Mean number of months lived in 5 years from onset — Maximum possible	60·00	—	—
Mean number of months lived in 5 years from onset — Expected	58·44	—	—
Mean number of months lived in 5 years from onset — Actual	58·50	—	—
Per cent of Expected	100·10	—	—
Radium alone—50 cases.			
Number of cases of known duration	39	5	3
Mean number of months lived in 5 years from onset — Maximum possible	60·00	60·00	—
Mean number of months lived in 5 years from onset — Expected	54·90	51·80	—
Mean number of months lived in 5 years from onset — Actual	46·72	39·40	—
Per cent of Expected	85·10	76·07	—
Combinations of radium and X-rays—24 cases.			
Number of cases of known duration	18	3	3
Mean number of months lived in 5 years from onset — Maximum possible	60·00	—	—
Mean number of months lived in 5 years from onset — Expected	54·18	—	—
Mean number of months lived in 5 years from onset — Actual	46·11	—	—
Per cent of Expected	85·11	—	—

	All stages.
Not treated by surgery or by radiotherapy—25 cases.	
Number of cases of known duration	17
Mean number of months lived in 5 years from onset — Maximum possible	60·00
Mean number of months lived in 5 years from onset — Expected	54·40
Mean number of months lived in 5 years from onset — Actual	17·41
Per cent of Expected	32·00

These figures show that in Stage I hysterectomy with excision of tubes and ovaries gave the best results, which were not improved by the use of radiotherapy either before or after operation. The 5-year survival rate and the expectation of life after panhysterectomy alone in Stage I were both higher when operation was supplemented by radiotherapy, but the number of cases so treated was small. Supravaginal hysterectomy alone in Stage I gave a very poor survival rate, but a fair expectation of life which was increased by the use of radiotherapy in addition. Treatment by radium alone in Stage I gave a 5-year survival rate of 55 per cent as against 73·5 per cent after radical operation, but when estimated by actuarial methods the former showed a 5-year expectation of life of 85·1 per cent as against 89·07 per cent for the latter. The numbers of patients in Stages II and III who were treated were too small for the differences in 5-year survival rates or expectation of life between different methods of treatment to be significant.

Biopsy.

	No.		Per cent.
Curettage and biopsy done before treatment	89		
,, ,, ,, at commencement of treatment	71	185.	64·2
,, ,, ,, during radiation treatment	—		
,, ,, ,, but time not stated	25		
,, ,, not done	103		35·8

Pathological Report.

	No.	Per cent.
No histological examination before or after death	33	11·5
Histological examination done before or after death	255	88·5
The specimen examined was non-malignant	1	—
Result of histological examination was doubtful	4	—
Adenocarcinoma	173	—
,, papillary	26	—
Spheroidal cell carcinoma	4	—
Squamous cell carcinoma, keratinizing	6	—
,, ,, ,, non-keratinizing	1	—
,, ,, ,, undifferentiated	3	—
Chorion-carcinoma	2	—
Carcinoma, type unspecified	17	—
Sarcoma	14	—
Spindle cell sarcoma	3	—
Leio-myosarcoma	1	—

Degree of invasion found on histological examination.

	No.
Growth confined to endo metrium of corpus uteri	16
,, had invaded the muscle ,, ,,	58
,, ,, spread to peritoneum	8
,, ,, ,, cervix	10
,, ,, ,, tubes or ovaries	12
,, ,, ,, pelvic lymph nodes	8
No pathological report on degree of extension	138

The two patients with chorion-carcinoma were aged 25 and 27 years respectively. One had borne no children but had had a miscarriage two years before admission; the other had borne a child four and a half months previously. The latter patient is still alive, the former died with metastases soon after admission.

Relationship between sarcoma and fibromyoma.

Amongst the 250 tumours which were microscopically examined there were 14 round cell or pleomorphic sarcomata, 3 spindle cell sarcomata, and one leiomyosarcoma. The incidence of fibromyoma amongst these 18 patients was as follows:

	No.
Sarcoma arose in a degenerating fibromyoma	9
There was a history of previous fibromyoma	2
,, no ,, ,, ,, and none was present	5
Not stated	2

Basis of diagnosis in 288 cases.

Clinically malignant:

	No.	Per cent.
Proved malignant by histological examination and/or autopsy	251	87·2
,, ,, appearance or metastases of recurrence	13	4·5
Diagnosis based on clinical evidence only	12	4·2

Clinically benign:

Proved malignant by histological examination	12	4·2

Other Primary Growths.

Previous primary tumours—4 patients.

1. Age 57. Breast amputated three years previously for cancer; no sign of recurrence. Admitted for columnar adenocarcinoma of uterus; treated by panhysterectomy with salpingo-oophorectomy. Died from abdominal metastases in the second year.

2. Age 78. Treated by X-rays two and a half years previously for cancer of the left breast. Diagnosed by biopsy of an axillary node; no sign of recurrence. Admitted for columnar cell carcinoma of uterus; treated by radium. Died from cancer in the third year.

3. Age 67. Breast amputated three years previously; no sign of recurrence. Admitted for adenocarcinoma of uterus, treated by radium and X-rays. Died three months later from metastases in the liver and abdominal organs.

4. Age 59. Radical mastectomy for spheroidal cell carcinoma of breast nine years previously, no sign of recurrence. Admitted for cancer of uterus (no histology); treated by radium; recurrence one year later and death from generalized metastases.

Simultaneous primary growths—6 patients.

5. Age 68. Admitted for columnar cell carcinoma of uterus; treated by panhysterectomy with salpingo-oophorectomy. A tumour in the left breast was found on examination, diagnosed as cancer, and treated by radical mastectomy. The patient died in the first year from pelvic metastases.

6. Age 63. Admitted for adenocarcinoma of uterus co-existing with a fibromyoma which had been present for many years. Treated by panhysterectomy with salpingo-oophorectomy. A tumour in the left breast was found on examination, diagnosed as cancer, and treated by X-rays. The patient died in the fourth year from metastases in the lungs, probably arising from the breast tumour.

7. Age 78. Admitted for cancer of uterus of about three years' standing. Found to have a tumour in the left breast, diagnosed as cancer. Died soon after admission.

8. Age 60. Admitted for adenocarcinoma of uterus; treated by panhysterectomy with salpingo-oophorectomy. Found to have a rodent ulcer of face of five years' standing; no note as to treatment. Died in the third year from recurrence of carcinoma in the pelvis.

9. Age 61. Admitted for columnar cell adenocarcinoma of uterus; treated by panhysterectomy. Found to have a rodent ulcer of the nose, which was treated by X-rays. Alive with no sign of recurrence of either growth after five years.

10. Age 60. Admitted for adenocarcinoma of uterus; treated by panhysterectomy and salpingo-oophorectomy. Metastases found in right ovary. Found to have a growth in right breast, diagnosed as cancer, for which treatment was refused. Not traced.

Subsequent primary growths—one patient.

11. Age 66. Admitted for columnar cell carcinoma of uterus of four months standing; treated by radium followed by panhysterectomy. One month later a growth in the right breast was found on biopsy to be a highly malignant spheroidal cell carcinoma. No treatment for the breast growth. Died in the first year from the growth in the uterus.

Cause of Death in 143 *Patients.*

	No.
Cachexia	97
Cardio-vascular disease	8
Pulmonary complications	8
Uraemia	5
Peritonitis	5
Intestinal obstruction	3
Haemorrhage	3
Pulmonary embolism	3
Sepsis	2
Surgical shock	1
Cerebral embolism	1
Intercurrent disease or unknown cause	7

Autopsy.

	No.	Per cent.
No autopsy	108	75·5
Autopsy done	35	24·5

Relevant autopsy findings (multiple in some cases).	No.
Local growth only	5
Extension to neighbouring parts	15
Metastases in pelvic lymph nodes	6
,, abdominal lymph nodes	8
,, liver and abdominal organs	11
,, lungs and pleurae	11
,, brain	1
,, skeletal system	1
Pulmonary complications	8
Abdominal ,,	5
No growth found (operated cases)	7

RECURRENT CASES.

There were 33 recurrent cases, of which 21 followed panhysterectomy (with salpingo-oophorectomy in 4 cases) for cancer, 2 followed hysterectomy for fibroids, and 10 followed radium treatment for cancer. The relation of the type of recurrence to the method of treatment of the primary and the mean intervals of freedom are shown below :

Following panhysterectomy for cancer—21 cases.	No.	Mean interval of freedom in months.
Local recurrence only	4	16·7
Recurrence deep in pelvis	9	19·7
,, both local and in pelvis	2	3·5
Distant metastases	6	16·2

The shortest interval was two months and the longest four years.

Following hysterectomy for fibroids—2 cases.		
Recurrence deep in pelvis	2	125

In both these cases the diagnosis of malignancy was made on the histological examination. One operation was a total hysterectomy, followed by 15 years of freedom from recurrence, and one was a subtotal hysterectomy for a fibromyoma, reported histologically to be suggestive of sarcoma, and followed by freedom for nearly 6 years.

Following radium treatment for cancer—10 cases.	No.	Mean interval of freedom in months.
Recurrence deep in pelvis	6	13·0
,, both local and in pelvis	1	18·0
Distant metastases	3	25·7

The shortest interval was five months, and the longest five years.

There were histological reports, either on the primary or on the recurrence, in 17 cases: Three sarcomata, 2 undifferentiated carcinomata, and 12 adenocarcinomata.

Fifteen patients received further treatment: by X-rays in 9 cases, radium in 4, radium with X-rays in one, and by hysterectomy followed by X-rays in one patient in whom the primary had been treated by radium. The latter patient was the only one of the 33 who survived five years.

SUMMARY.

1. A statistical analysis of 321 cases of cancer of the corpus uteri. 288 of these were primary cases.

2. 22·9 per cent of the 288 primary cases were single women and 77·1 per cent were married or widowed. The mean age of the 66 single women was 58·3 years, and that of the 222 married and widowed 58·6 years.

3. Analysis of the ratios of married and widowed to single women by age groups (cervix uteri Part I, page 434) led to the conclusion that liability to cancer of the corpus uteri is somewhat less among married and widowed than amongst single women between the ages of 35 and 65.

4. There were 99 nulliparous patients in all, and amongst 202 married and widowed patients of known parity there were 44 nulliparae against 33 expected (calculated from the percentages of childless married and widowed women in each age group registered as dying from all causes in England and Wales in 1939), indicating a greater liability to this form of cancer amongst nulliparous than amongst parous married women.

5. 5·2 per cent of the patients gave a history of having suffered from uterine fibromyomata, and in 10·4 per cent the patient was found to be suffering from both conditions simultaneously.

6. Irregular haemorrhage was the first symptom in 63·2 per cent of the patients, vaginal discharge in 21·9 per cent.

7. Only 33·7 per cent of the patients consulted a doctor within three months of noticing the first symptom, 14·2 per cent within the next three months, and in 39·6 per cent the symptoms were of more than six months' standing before advice was sought; in 17 cases the delay was more than two years.

8. In 76·7 per cent a vaginal examination was made at the first consultation, and in a further 6·9 per cent it was done within one month of the first consultation. In only 4·3 per cent was the examination delayed longer than one month.

9. 73·7 per cent of those who consulted a doctor were referred to hospital forthwith, 3·8 per cent were treated symptomatically for periods up to three months before reference, and 6·6 per cent for longer periods, but this figure included patients who were unsuitable for active treatment by reason of poor general condition or of the disease being too advanced. In 2·3 per cent the patient was told that the symptoms were due to the menopause.

10. On admission to hospital it was found that in 65·6 per cent of the patients the disease was still confined to the uterus, in 15·3 per cent it had spread to the parametrial tissues or to the cervix or Fallopian tubes, in 9·7 per cent the regional nodes, bladder or rectum were involved, and in 9·4 per cent there were metastases outside the pelvis.

11. Seventy-four patients were treated by panhysterectomy with bilateral salpingo-oophorectomy, with an operative mortality of 5·4 per cent and a five-year survival rate of 73·5 per cent of traced cases in Stage I, 44·4 per cent in Stage II, and 33·3 per cent in Stage III. Estimated actuarially, the five-year expectation of life was 89·07 per cent of normal expectation for Stage I, 74·5 per cent for Stage II, and 53·6 per cent for Stage III.

12. In 12 patients panhysterectomy with bilateral salpingo-oöphorectomy was preceded by radium treatment, with a five-year survival rate of 75·0 per cent, and in 22 it was followed by radiotherapy, with a five-year survival rate of 63·2 per cent of traced cases. The five-year survival rates showed that there was definite improvement in the results in patients who were classed as Stage II and III, but no improvement in those in Stage I. The numbers so treated were too small for the results to be statistically significant.

13. Twenty-two patients were treated by panhysterectomy alone, with an operative mortality of 22·7 per cent and a five-year survival rate of 46·6 per cent in Stage I. When the operation was preceded by radium treatment, 75·0 per cent of four patients survived five years, and when it was followed by radio therapy, 77·7 per cent of nine patients survived five years. These numbers also were too small for the results to be statistically significant.

14. Eighty-six patients were treated by radiotherapy alone, with five deaths due to effects of treatment (5·8 per cent) and a five-year survival rate of 43·0 per cent.

15. Twenty-five patients were not treated either by surgery or radiotherapy; all are dead after an average duration of life of 17·4 months.

16. Biopsy was done in 64·2 per cent, and there were histological reports for 88·5 per cent of the patients.

17. There were 21 patients with recurrent growths following panhysterectomy for cancer, two following hysterectomy for growths diagnosed clinically as fibroids, and ten following radium treatment for cancer. Fifteen of these patients received further treatment, 14 by radiotherapy, and one, whose primary growth had been treated by radium, had hysterectomy performed; the latter patient was the only one who survived five years.

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THE STATUS OF GENETICAL STUDIES IN HUMAN CANCER.

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A PROBLEM fundamental in research on human cancer is the question whether there are causative factors common to cancers of different sites, and, if so, to what extent. It would speak in favour of the existence of such factors if it were possible to demonstrate an increased tendency to cancer among male relatives of women with cancers of specifically female type.

In " Family G " described by Warthin (1925) 15 out of 23 cancers in women were adenocarcinomata of the endometrium, while all the 20 cancers in men were localized to the stomach or intestine (Hauser and Weller, 1936). Wassink (1935) has also produced results pointing in this direction, but the solution of the problem mentioned will demand further evidence.

Publications in recent years on the heredity of human cancer display some divergence of results, which may appear bewildering to the student of cancer interested in the problem but unfamiliar with genetics. Furthermore, it is sometimes forgotten that the difficulties encountered in this field are not only genetical but also clinical and sociological, so that conclusions reached by geneticists in apparent safety may become endangered through progress in clinical technique and sociology. Even quite extensive work has sometimes resulted only in this knowledge, and consequently in the obligation to pass it on. For these various reasons a synthesis of some recent publications on the heredity of human cancers will be attempted here.

In 1942 the University Institute for Human Genetics and the Danish Cancer Registry, both in Copenhagen, entered into collaboration to examine the extent to which some types of human cancer were inherited, thus exploiting the unusual opportunity afforded by a small population under registration for the disease in question, uniform and well-suited for the purpose, both mentally, socially and racially, all members having equal access to first-class medical facilities.

The publication of the results from these studies was followed by contributions from other authors, in some cases of a critical character, and the whole complex of monographs and articles will briefly be reviewed here.

The choice of cancer types for the investigation will serve to illustrate the character of the task, and the difficulties in evaluating results. It is vital that the cancer chosen is accessible to the establishment of a diagnosis with some certainty, not so much with regard to propositi themselves as with a view to their relatives, the diagnoses of whose cancers have often been made years ago and under less favourable conditions—a factor far too often overlooked in studies of this kind. Consequently, the abdomen and the lung were out of the question as sites for the investigations planned.

Prognosis is also of importance. Cancer of the skin is unsuited, because the easy treatment and favourable prognosis leave the patient and his relatives

ignorant of the severity of the affection, and thus forgetful about its existence. The high age of onset of this disease is also a disadvantage, because relatives of patients being older on the average will have had their diagnoses made some decades earlier, and thus under circumstances different from the present.

An unfavourable prognosis may, on the other hand, fatally diminish the number of patients available for an interview. Oesophageal cancer presents this difficulty, combined with high average age and often with poor social conditions. Dr. Mogensen, otologist, made a heroic effort in collecting 75 families in spite of all difficulties, but met with the ultimate obstacle that heavy drinking is very common, not only among the later patients, but also among their relatives. So even a pronounced familial occurrence of oesophageal cancer might be due to the inheritance of the mental qualities of habitual drinking, which habit, directly or indirectly, is associated with the development of oesophageal cancers.

Rectal cancer was deemed unsuited for investigation because of the difficulty in excluding the influence of inheritable intestinal polyposis on the development of cancers in this site.

Thus mammary cancer (Jacobsen, 1946), leukaemia (Videbæk, 1947) and the uterine cancers of cervix and corpus (Brøbech, 1949) were chosen for the investigation. It was an advantage that all these affections have an early age of onset, but it had to be realized that the differential diagnosis of the latter three diseases will often be less firmly established for relatives than for propositi themselves.

The technique of the three investigators was identical in principle, although some improvements were introduced in the course of their studies. About 200 patients were, as a rule, interviewed by the investigator in person about their relatives, their data and diseases. Since birth dates are always known in the Danish population, it was possible to see to it that the age distribution of propositi corresponded closely to the distribution of all cases in the country. A corresponding number of carefully selected normal persons, as far as possible of corresponding age, etc., were likewise questioned. All diagnoses of cancer and of many of the remaining fatal cases were, as far as possible, confirmed through death certificates or case records from hospitals. This applies, for instance, to all cases of cancer among close relatives of Jacobsen's breast cancer patients. Detailed pedigrees are given for all patients with mammary cancer and leukaemia in Jacobsen's (1946) and Videbæk's (1947) monographs, so that every case can be easily checked.

With some aid from Jacobsen these possibilities for further studies have been exploited for a critical review by Busk (1948), and Busk, Clemmesen and Nielsen (1948). Busk's (1948) computations are based on the idea that control persons will never take quite the same interest in cancer cases among their relatives as do patients and their families, to whom the question about the inheritance of cancer has become topical. However, mortality figures will be serviceable as a " yardstick " comparable to the memory of relatives, which will most often be limited to fatal cases, so that the more correct figures from a cancer registry will be less useful. Exceptionally, Busk has replaced mortality figures for mammary cancer by the Cancer Registry's figures for incidence, because of the assumption that this affection would be regarded by Danish laymen as a dangerous but not incurable affection to be remembered in the family even if cured, while natural shyness would prevent uterine cancers from being discussed and remembered in the same way, so that mortality figures suffice for the latter as for the remaining sites.

On this basis it was possible to compute the number of cancers expected in each group of relatives with full allowance for the variation in age distribution. For relatives to control persons, Busk (1948) computed that Jacobsen (1946) had succeeded in finding only 81, or 29 per cent, out of 278 cancer cases expected among siblings and parents, and parents' siblings and parents. Videbæk (1947), benefiting from Jacobsen's experience, found 218 out of 300, or 73 per cent, presumably because he systematically interviewed more than one person in each family. For parents and siblings, however, Videbæk's figures for normal families show good correspondence with the expected values, and consequently we shall limit ourselves to such conclusions as can be drawn from the findings among siblings and parents of patients, termed " close relatives," since there seems to be justification for accepting the " yardstick " as practically valid for these categories.

Mammary Cancer.

From Jacobsen's (1946) review of previous studies it appears that results interpreted as positive with regard to inheritance of mammary cancer date as far back as one century, but even in the last two decades authors of reputation (Stocks and Karn, 1933 ; Passey, 1942 ; Hanhart, 1943) find no evidence that a history of cancer in relatives increases the risk of cancer to any measurable extent.

An important reason for doubting the positive results was the discussion about the validity of control materials as an expression of the full number of cancers occurring among relatives of normal persons. However, by Busk's (1948) computations on Jacobsen's (1946) results it has been demonstrated that close relatives of patients with mammary cancer show a significantly increased number of cancer cases. This result is beyond doubt, since all the cases among close relatives have been confirmed through hospital case records or death certificates, and since the control figures for breast cancer itself have been computed from real incidence figures, which, as will be seen later, make a severer test that mortality figures.

It will appear from Table I that there is not only an increase in the incidence of mammary cancer among the female relatives of the patients, but also a significant increase in the incidence of cancers at other sites among mothers, while sisters show a tendency in the same direction. Male relatives also display a significant increase of cancers.

Thus Jacobsen's (1946) two main theses, (1) on the increased incidence of mammary cancer, and (2) on the increased incidence of other cancers among the relatives of breast cancer patients, have been borne out by his first critics, but further evidence on the subject has been brought forward by Smithers (1948), and by Penrose, Mackenzie and Karn (1948*b*).

Smithers (1948) reported on 459 family histories of patients suffering from breast cancer. The histories had been taken at the time of the patients' first visit to hospital, and had at the time of publication not yet been supplemented by later questions to propositae or relatives. Apparently the material was not collected by the same investigator, but Smithers, who does not overestimate the value of his material, rightly thinks it worthy of notice that the figures show an unduly high incidence of cancer of the breast, and *not of other cancers.*

Some part of Smithers' (1948) material is included in the figures given by Penrose *et al.* (1948*a*), of which those for close relatives are quoted in Table I.

TABLE I.—*Incidence of Cancer Cases in Relatives of Patients and Control Persons.*

	Patients' relatives.				Control families.			
	Breast.		Other sites.		Breast.		Other sites.	
	Observed.	Expected.	Observed.	Expected.	Observed.	Expected.	Observed.	Expected.
Jacobsen (1946); Busk, (1948):								
260 Mothers	21‡	7	34*	22	200 Mothers	2	4	17	23
381 Sisters	13‡	5	17	11	433 Sisters	2	2	6	10
199 Fathers	1	..	39‡	23	199 Fathers	..	..	19	22
377 Brothers	..	..	16*	8	389 Brothers	..	..	4	10
Penrose, Mackenzie and Karn (1948*a*):								
406 Mothers	25	11·12	51	49·23				
307 Sisters	23	6·97	19	25·23				
420 Fathers	..	0·10	41	52·32				
455 Brothers	..	..	29	28·14				

* Signifies that the probability of the difference found, under the assumption that the true incidence was the same for the two materials compared, amounts to between 5 and 1 per cent.

‡ Signifies correspondingly a probability below 1 per thousand.

TABLE II.—*Incidence of Cancer Cases in Relatives of Patients and Control Persons.*

	Patients' relatives.				Control families.			
	Uterus.		Other sites.		Uterus.		Other sites.	
	Observed.	Expected.	Observed.	Expected.	Observed.	Expected.	Observed.	Expected.
Brøbech (1949):								
Carcinoma of the cervix.								
200 Mothers	6	3·0	22	20·3	200 Mothers	6	3·1	19	21·1
488 Sisters	10†	2·2	12	7·7	449 Sisters	1	2·1	8	7·6
200 Fathers	..	..	26	19·7	200 Fathers	..	..	22	19·5
495 Brothers	..	..	9	8·2	423 Brothers	..	..	4	6·6
Carcinoma of the body of the uterus.								
90 Mothers	6*	1·7	16	13·3	90 Mothers	3	1·7	12	14·0
217 Sisters	6*	1·8	11	7·7	233 Sisters	1	1·8	10	8·0
90 Fathers	..	..	18	13·0	90 Fathers	..	..	10	11·9
184 Brothers	..	..	8	6·5	212 Brothers	..	..	6	6·0

	Cancer all sites.			Cancer all sites.	
	Observed.	Expected.		Observed.	Expected.
Videbæk (1947); Busk (1948): *Leukaemia.*					
208 Fathers	31*	20	200 Fathers	18	19
457 Brothers	18	15	393 Brothers	9	14
209 Mothers	28	24	200 Mothers	21	22
447 Sisters	32†	18	409 Sisters	15	17

* As in Table I.
† Signifies a probability between 1 and 0·1 per cent.

It will be seen that, while there is no doubt of familial transmission of breast cancer, which for the first time is demonstrated on English material, no such phenomenon is encountered in this material for cancers of other sites. This will be discussed following a review of other publications.

A minor but significant result from Busk's (1948) studies on Jacobsen's (1946) figures is the demonstration that breast cancer, when occurring in related persons, is preferably located to the same side in persons closely related. This observation, which leads to the final confirmation of the higher frequency of cancer in the left breast (Busk and Clemmesen, 1947), has been confirmed by Penrose *et al.* (1948*a*, *b*), but it does not necessarily mean that the tendency to cancer is inherited. The present author would think that a familial difference in the quantity of glandular tissue, of a purely anatomical nature, would explain the phenomenon.

More positive evidence in favour of the inheritance of mammary cancer might be seen in the fact that Jacobsen (1946) found a heavy taint in all of six families with cases of male breast cancer. However, Penrose *et al.* (1948*a*) found nothing noteworthy in the family histories of their two male cases.

It is instructive to see the various opinions as to the so-called anticipation or earlier occurrence of cancers among later generations of tainted families. Busk (1948) thinks that Jacobsen's (1946) and Videbæk's (1947) results pointing in this direction are due to the imperfect information from old propositi about their relatives, often dead for many years, which makes it more difficult to demonstrate a taint in the pedigree of older propositi. Penrose *et al.* (1948*b*) and Smithers (1948), who find a slightly higher average age for their propositi with familial taint, explain that patients under observation in the younger age groups are not likely to have many sisters old enough to have developed the disease. Either way, anticipation or the opposite seems easily ascribable to technical sources of error.

Uterine Cancers.

Recently, Brøbech (1949) has published a comparative study of the uterine cancers of cervix and body, comprising respectively 200 and 90 patients with siblings and parents and their parents' siblings and parents. Comparisons are made with the cancer incidence in 200 control families, 112 of which have been selected from Videbæk's (1947) control families, and besides, the expected cancer incidence among the various groups of relatives is computed according to Busk's (1948) method.

Brøbech's (1949) control families show the expected values for cancer cases among close relatives, so that comparisons for these groups seem as reliable as can be, but even so the views given with regard to the value of questioning about relatives more remote than parents and siblings apply also to Brøbech's material.

Cervical cancer.

It is seen from Table II that sisters of cervical cancer patients show a significant increase of uterine cancer as a whole, but not of cancers of other sites. Fathers show a corresponding, isolated and significant increase of oesophageal cancers. Evidently there is no significant increase of cancers of other sites, nor among male relatives, as in Jacobsen's (1946) material, and Brøbech (1949) questions the influence of hereditary factors on cervical cancer. It seems more

likely that the increased incidence of cervical cancers would be ascribable to exogenous factors, since sisters are more likely to have the same sexual behaviour than mothers and daughters. When Brøbech considers the possibility that the combined increase of cervical and oesophageal cancer might be conditioned by a tendency to cancers of squamous epithelium, it should be stressed that extrinsic factors are almost bound to affect this epithelium. Furthermore there might be an explanation in the fact that both these types of cancer are commonest in the lower social classes.

Cancer of the body of the uterus.

Owing to the rarer occurrence of this type of cancer Brøbech's material comprises only 90 patients. Still, mothers and sisters of patients both display a significant increase, but for fathers and brothers there is only an insignificant tendency to increased cancer incidence.

Leukaemia and cancer.

Videbæk, whose monograph followed Jacobsen's, worked out his own material of normal families, improving the technique used by the latter. It appears from Busk's calculations that there is a significantly increased incidence of cancer among fathers and sisters of leukaemia patients.

Naturally, the question arises whether this involves an increase in cancer caused by inheritance, although the possibility may be doubted, because the taint is unevenly distributed, compared to Jacobsen's findings or Brøbech's for cancer of the uterine body.

DISCUSSION.

It is a point of general agreement among recent authors that mammary cancer is familially transmitted. The latest English authors, Penrose *et al.* (1948*a*, *b*) and Smithers (1948), following Wassink (1935) and Jacobsen (1946) in accepting the inheritance of this type of cancer, have finally justified the large number of earlier records of families with several cases of mammary cancer. But here ends uniformity, and a very important divergence appears on the issue whether cancers of other sites are increased in frequency among relatives of patients with carcinoma of the breast.

In 1935 Wassink reported on such an increase in frequency for relatives of breast cancer patients in comparison with relatives of patients with uterine cancer. Jacobsen found an increased tendency to cancer of other sites among relatives to breast cancer patients, significant for three of the four categories of close relatives, but Penrose *et al.* and Smithers found no increased occurrence.

In estimating results it should be remembered that we are dealing with figures not only suffering from the ordinary inaccuracy of biology but based on clinical investigations, the results of which have sometimes been reflected through the memory of relations to patients.

A second qualification to be borne in mind is that, in comparing the incidence found among relatives of patients with the mortality figures of the general population, we cannot be quite certain of that uniformity between the groups compared which forms the fundamental basis of statistical comparison. It is true that without knowing the computed values both Videbæk (1947) and Brøbech

(1949) succeeded in obtaining correspondence between observed and computed numbers of cancer cases, so that the computed " yardstick " seems well comparable with the results from inquiry in normal families as far as close relatives are concerned. But it may be that both values are equally too low, one being based mainly on mortality statistics, and the other on information from normal families where no recent case among close relatives has turned attention to the problem of the inheritance of cancer.

Thus, when Videbæk finds a significantly increased incidence of cancers among fathers and sisters to leukaemia patients, and some tendency in the same direction for mothers and brothers, it seems to support Jacobsen's results. But the support is weakened when Brøbech finds a similar tendency for all close relatives of cases of both cervical and uterine body cancer, and the question arises whether the tendency demonstrated is not due to differences in the memories of the families concerned, so that discussion should be based only on differences that are statistically significant. The fact that Wassink found a far lower incidence among relatives of uterine cancer cases than in the families of patients with breast cancer seems to point in this direction.

Thirdly, it is obvious that in abandoning the material of relatives remoter than siblings and parents of patients—and this we must do—we destroy our only possibility of estimating the path of inheritance and at the same time our means for excluding the effect of " false inheritance," i.e. of environmental factors in the home or social level of the closer family. The importance hereof will be evident from the following.

For sisters to patients with cervical cancer and for their fathers there was a significant increase of uterine and oesophageal cancer respectively, but neither mothers nor brothers were affected to any degree of statistical significance, and both types of cancer are known to vary in frequency with social conditions. If we assume that, in spite of all precautions, Brøbech's (1949) propositae were of a lower social class than his control persons, this might account for the increase in both cases. It is difficult to avoid this hypothesis when Videbæk (1947, p. 26) mentions that he had to choose such control persons as could be made to take an interest in the work, and found them in the University staff and among his own acquaintances, and when it is remembered that Brøbech took over from Videbæk 112 out of 200 families, and from Brøbech's own statement it is clear that his patients with cervical cancer were poorer than the average population.

Also, the difference between cancer frequency among relatives of the uterine body and cervical cancers may, of course, be apparent only, but since there is other evidence that, in the population concerned, cervical cancer is far more dependent on social factors than cancer of the body, it is justifiable to believe that the more even taint among the female relatives by the latter disease is more likely to express a genuine taint of hereditary character than the isolated highly significant increase for sisters of cervical cancer patients.

Thus it is evident that when the frequency of cancer of a certain site varies between the social classes we shall find it difficult to demonstrate a possible hereditary tendency, which, after all, may be the reason why the individual develops a cancer as a response to the carcinogenic factors of his social setting. In future it should be demanded in genetical studies that full attention is paid to the influence of environmental factors and social conditions of propositi.

It is a rule, too often violated in cancer sociology, that we should not suggest hypothetical pathogenic factors as long as we can explain findings through factors of well-known causative influence. Consequently to the deliberations above, differences in race or even in social conditions should rank second to technical differences in material and procedures as an explanation of the very important difference between Dutch-Danish and English results with regard to the incidence of other cancers among relatives of breast cancer patients.

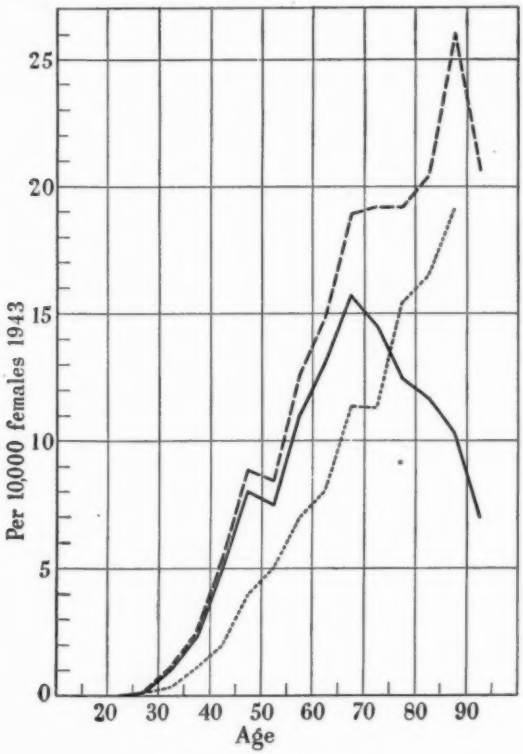

FIG. 1.—Cancer mammae.

——— Notified cases (hospitals).
– – – – – Notified cases plus deaths, previously not notified.
..... All fatal cases (certificates).

As to procedure, there is no doubt that the English workers have chosen a more correct way than the Danish in comparing their findings with mortality figures from the period in which occurred the deaths of the relatives concerned even as far back as in the past century. On the other hand, the Danish computations were based on modern figures, which give a more cautious comparison because breast cancer has been increasing in frequency during recent decades, presumably in conjunction with the declining birth rate. Incidence figures, as used by Busk (1948), are at a still higher level, as will be seen from Fig. 1, in

which the curves for real incidence are compared with mortality figures, both distributed by age. The difference between the curves is exaggerated because the hospital cases are grouped according to age when first seen in hospital, while deaths are classified according to age at death, so that this fictitious difference will also influence computations. Which of the two " yardsticks " will correspond best to the memory of the public with regard to cancer frequency among relatives will, of course, be a matter for conjecture, but there is no doubt that Jacobsen's material by Busk's treatment has passed a severer test than that of Penrose *et al.*

Jacobsen's procedure in analysing the site of cancers found among relatives of his patients is, however, scarcely defensible. Between registration material and mortality statistics there is a wide difference in the percentage of sites, caused by the differences in prognosis, and it will at times be very difficult to evaluate a deviation from the percentage expected. Furthermore, it is questionable which sites of cancer will be best known in the family when the question is about remote relatives, so that Brøbech's attempt at computing the value corrected for imperfect information from remoter relatives will be without much interest. Fortunately the deviations in his percentage of oesophageal cancer are so pronounced that they, within their limits, must be considered as valid.

In the mere technical procedure, it is thus difficult to find any reason why Jacobsen's increase of male and other cancers among relatives to breast cancer patients should not be demonstrable on an English material, apart from Jacobsen's advantage of working personally and within a smaller population.

With regard to the social distribution of cancer, it is clear from the publications of the Registrar-General that social classes in England prevent pronounced differences in cancer mortality. How far these differences will influence an investigation such as that of Penrose *et al.* it is difficult to decide, but there are two theoretical possibilities.

It is known that mammary cancer is most frequent among the higher social classes, while many other cancers, especially those of supposed exogenous origin, are frequent among the poorer. In choosing propositae for a genetical investigation into mammary cancer two things may happen. Provided we avoid selection completely, the average patient with breast cancer will probably be from a higher social class than the average proposita. Consequently, her relatives, especially males, will show a lower incidence of all cancers, and we may get a result which, compared with Jacobsen's, shows just the differences displayed by the material of Penrose *et al.* If, on the other hand, facilities for research work are best in the less well-to-do strata of the population, we shall still be able to acquire the number of propositae necessary for our examination, but their relatives will show a larger number of cancers, especially extrinsic forms, than that expected from the average.

The principle of these deliberations demonstrates that with the information at hand it is impossible to judge the differences between the materials of Jacobsen and Penrose *et al.* But it is a fact that the latter have been able to demonstrate an increased frequency of mammary cancer among relatives, which was not demonstrable only a few years ago, by finding more cases among relatives than the earlier investigators. Assuming the correctness of all the statements concerned, it is only natural to expect a confirmation of Wassink's and Jacobsen's results.

Meanwhile it will not be superfluous to stress the necessity that genetical investigations in cancer pay as full attention to differences in social distribution as to differences in age incidence. But difficulties in tracing relatives with tumours make cancer registration a fundamental basis to such investigations. While therapy makes progress, computations based on mortality statistics will become increasingly dangerous as a substitute for controls, and probably modern death certificates are already less reliable than Wassink's in 1935, so that his method of comparison of cancers of different sites may still be useful.

Further investigations with a view to testing the theory of combined inheritance of cancers of different sites will still be of interest, even if they have to be carried out on a type of cancer influenced by hormones like mammary cancer. Also Brøbech's observations on cancer of the uterine body are a stimulus to further studies, although subject to the same qualification. Studies of cancer in twins may be of value, too, especially if they could be carried out with the same efficiency as recent American twin studies on tuberculosis by Kallmann and Reisner (1943), but on the whole the field of genetics in human cancer will necessarily be strictly limited until cancer registration, which forms the only solid base for such research, can gain ground.

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MENINGEAL TUMOURS WITH EXTRACEREBRAL METASTASES.

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INTRACRANIAL tumours seldom appear to metastasize, apart from the tendency of the medulloblastoma to meningeal spread in the spinal canal. Extracerebral metastases are still more rare, and this applies both to meningeal tumours and gliomas. It is difficult to give a satisfactory explanation of this, but the cause may be that especially good conditions are required for the growth of these tumours and that these conditions are found within the skull. This contention is supported by the fact that cerebral metastases occur frequently with extracranial tumours.

In the literature there are only a few verified cases of extracerebral metastases from meningeal tumours. It is, therefore, of interest that within two months we have had the opportunity to see two patients with meningeal tumours with extracerebral metastases. These two cases are described in detail, and are compared with the five well-established cases found in the recent literature.

CASE HISTORIES.

Case I (*Record No.* 1078/48).

The patient was a single male, born May 20, 1911. His illness started in 1925 with universal epileptic fits which lasted for three years, and these were treated with barbiturates. There were no fits from 1928–32, but after that time left-sided sensomotor Jacksonian fits developed, beginning in the left arm. In 1933 the patient was admitted to the Neurological Department of the State Hospital, and from there moved to the Surgical Department D of the State Hospital, where craniotomy was performed with a total extirpation of a macroscopically typical meningioma over the right motor region. The tumour was well demarcated, and measured $6 \times 7 \times 4$ cm. It was finely lobulated and flattened, except anteriorly, where it was thicker and indented the underlying brain. It was of a tense, elastic consistence and of a yellowish-white colour. The histological diagnosis was sarcoma alveolare.

After operation he had nine X-ray treatments, and there were no immediate post-operative complications, but in spite of treatment with barbiturates the patient had, after an interval of 1–2 months, left-sided Jacksonian fits, beginning in the left arm and sometimes accompanied by unconsciousness. Finally a permanent left-sided paresis developed, invaliding him. In 1940 he was again admitted to the Neurological Department of the State Hospital, and from there moved to the Neuro-Surgical Department on February 20, 1940.

On clinical examination the left pupil was found to be bigger than the right, and reacted on convergence. There was left-sided and central facial paresis and slight paresis of left upper extremity. The left hand was pale and the hand

muscles atrophied. In the left leg the reflexes were exaggerated and ankle clonus was present. Astereognosis, dysmetry and dysdiadokokinesis were present in the left hand. Encephalography showed a shift of the ventricular system to the left, and a depression at the top of the central part of the right ventricle. At operation on March 1, 1940, a typical meningioma weighing 62 g. was removed ; it was adherent to dura in the centre of the right sphenoparietal sinus.

Histological examination showed a highly cellular meningioma, which closely resembled the specimen taken from the operation in 1933 when they were compared side-by-side.

The post-operative course was uncomplicated, apart from a single left-sided Jacksonian fit. On discharge, 19 days after operation, there was still a slight paresis of the fingers of the left hand, which could not be stretched fully, astereognosis and reduced position-sense of the left hand and slight left-sided drop-foot.

After discharge he was treated with barbiturates in increasing doses, and during the following years he had, now and then, usually at night, epileptic fits with unconsciousness, especially when he had failed to take his medicine. By the middle of 1947 he suffered from left-sided Jacksonian fits every second or third day, with the result that he had to give up his work as a fitter. He was admitted to the Neurological Department of the Municipal Hospital on November 21, 1947, where encephalography showed a deformity of the right frontal horn with a depression at the top. He was moved to the Neuro-Surgical Department of the State Hospital on January 14, 1948, where the clinical examination showed medium left-sided paresis, but otherwise the condition was as in 1940.

On January 16, 1948, he had left-sided Jacksonian fits with unconsciousness followed by vomiting, turning of the head to the right, a loss of the pupillary reaction to light, right-sided dilatation of pupils and deep respiration. This was followed by coma, increasingly rattling noisy respiration and a lack of reaction to pricking. The right pupil was always bigger than the left and the corneal reflexes were reduced. Oxygen inhalations and saccharosis were given without effect, and he died on January 18, 1948.

Autopsy.

Autopsy showed a nodular thyroid gland with an enlarged lobe and small cysts on the cut surfaces.

Thin fibrinoid membranes were found on the right visceral pleura, and the lower lobe of the right lung was slightly granular and solid with scattered greyish-white pneumonic foci, but without tumour infiltrations. The bronchi were filled with a purulent exudate, and there was a slight, cylindrical dilatation of the peripheral branches in the lower lobes. No tumours were seen here either.

The liver measured 34 × 26 × 10 cm., and weighed 3800 g. The surface was granular. On the cut surface of the right lobe there was a round tumour, 15 × 11 × 11 cm. in size. There were several smaller ones with diameters varying from 1–6 cm. distributed in both lobes. The demarcation from liver tissue was sharp. In the main tumour many thin-walled vessels up to 4 mm. in diameter were found ; the cut surfaces were greyish with large yellowish streaks. The smaller tumours showed a more uniform, white cut surface without macroscopical vessels. The intermediary liver tissue showed no signs of cirrhosis (Fig. 1).

On the surface of the right kidney a white, rather soft, well-demarcated tumour 3 mm. in diameter was found. The remainder of the organs showed nothing of interest.

The brain was oedematous. In the old operation cavity a well-demarcated tumour of the size of a hen's egg was found, showing no tendency to invade the brain tissue (Fig. 2). The walls of the cavity were greyish, and at the bottom there were fresh blood-clots continuing into the ventricular system, with which the operation cavity communicated. The cavity occupied the former position of most of the gyri of the right parietal lobe and extended a little into the occipital lobe. The tumour was adherent to dura. It was solid, greyish, and like fish meat on the cut surface.

Histology of the tumour (Fig. 3) showed fibril-forming cells lying close to one another with big nuclei, rich in chromatin of varying forms, containing one or several nucleoli. Between the cells there were streaks or larger quantities of collagen connective-tissue fibrils. The tumour was rich in vessels, but the walls of these showed no abnormalities. Mitoses were not present. The diagnosis was fibrosarcoma. On comparing the specimens from 1933 and 1940 the same histological picture was seen, and the diagnosis of meningioma made in 1940 must be considered mistaken. There is, in fact, no difference between the histological picture of 1933, 1940 and 1948, and the metastases in the right kidney and liver (Fig. 4) are identical with the primary tumour. The big vessels in the liver tumour appear to be preserved liver veins.

Sections of the prostate show moderate glandular degeneration in the form of cysts, in which numerous desquamating epithelial cells as well as corpora amylacea are to be found. There is no sign of malignant change.

On the cut surfaces of the thyroid gland there are medium to very big roundish follicles containing ample, not vacuolated colloid, and with a lining of uniform, small, dark, cubal epithelium. The parenchyma is divided into rough lobes by trabecular connective tissue. These are the appearances of a nodular colloid goitre without histological signs of malignancy.

Summary of Case I.

The patient was a tailor, aged 38, who had suffered, since the age of 14, from left-sided Jacksonian convulsions sometimes accompanied by unconsciousness. At the age of 21 he was operated upon and a typical meningioma, overlying the right motor region, was removed. After operation his health improved but his symptoms recurred, and he was again operated on at the age of 31. On this occasion a typical meningioma, overlying the right spheno-parietal sinus, was removed. When he was 37 years old he suffered again from frequent left-sided Jacksonian convulsions, in spite of barbiturate treatment, and a stationary left-sided hemiplegia. So on January 14, 1948, he was once again admitted to the Hospital for operation, which, however, was not performed, as he died 4 days after admission during epileptic convulsions with unconsciousness, from which he could not be awakened.

Autopsy showed a recurrence of the tumour over the right motor region, a large and several smaller tumours in the enlarged liver, and, in addition, a little tumour in the surface of the right kidney. Microscopical examination showed the same picture in 1933, 1940 and 1948.

Diagnosis : Fibrosarcoma with metastases in the liver and the right kidney.

Case II. (*Malmö General Hospital. Pathological-Bacteriological Department No. 543/47*).

The patient was a married man born January 5, 1907, who had been well until the age of 29, when he suffered from an epileptic convulsion with deep unconsciousness which lasted about 15 minutes. On examination at the Medical Clinic from July 8 to 17, 1936, a doubtful Babinski's reflex on the right side was found, otherwise there was nothing abnormal. In December, 1936, he suffered from another epileptic convulsion beginning in the right leg. He was admitted to the Hospital in January, 1938. A protrusion of the right parietal bone was found, and corresponding to that a thickening 4 cm. long in the theca cranii, where the external lamina measured up to 7 mm. in thickness except in one place, where a thinning of the size of a pea was found. On neurological examination nothing abnormal was found except the doubtful right-sided, positive Babinski's reflex. He was moved to the Surgical Department, where, on February 8, 1938, a craniotomy over the left motor region was performed. Here a tumour was found on the inner side of the dura, reaching down to the mid-line, pressing the motor zone backwards and downwards. The tumour was shelled out. On the bone over the tumour some granulations were scraped away, which were suspected to be tumour masses (Fig. 5). Histologically the tumours were seen to be meningiomas with fibrous framework (Lindau).

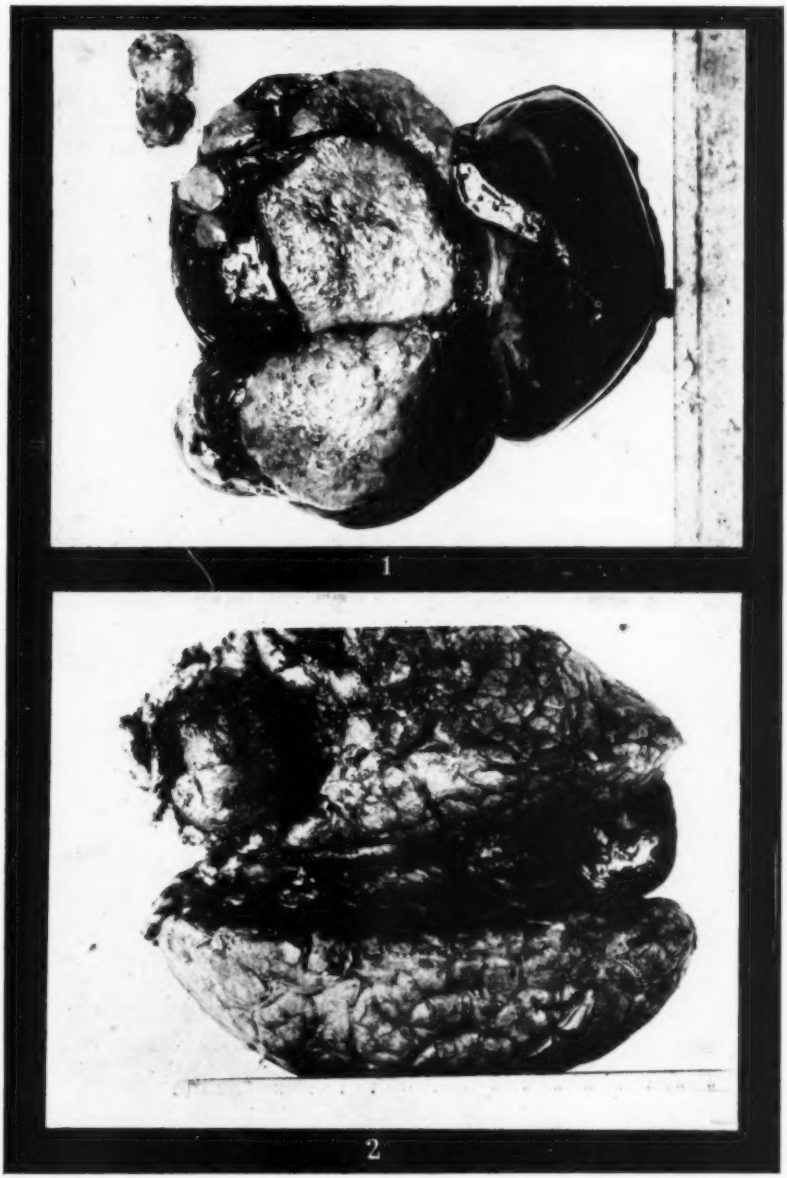
The post-operative development was uncomplicated, and on discharge from the Hospital the patient had no symptoms, but 8 months later he again had epileptic fits beginning in the right leg followed by unconsciousness. On re-admission to the Hospital 10 months after the operation he complained of head-ache, giddiness and weakness of the right leg, and there was slight exaggeration of the right-sided reflexes and an uncertain right plantar reflex. Luminal was given, and after that he improved so much that in 1939 he had only a few small epileptic fits, and from 1940–45 he was practically without ; on two occasions only he had small jerks in the right leg without accompanying unconsciousness.

In January, 1946, he had pains in the left side of the chest, especially on breathing deeply, a slight irritating cough and a transitory rise of temperature. X-ray photographs of the lungs showed a well-demarcated shadow 5 cm. in diameter on the left side. On auscultation in March, 1946, impaired air entry was found on the left side between the second and fifth ribs in the anterior axillary line, and in this region friction rubs were heard. Though no primary tumour could be found it was considered that there were probably multiple metastases in the lung, so the patient was treated with X-rays. A later X-ray photograph in October, 1946, showed expansion of the shadow in the left lung. The patient could do his work the following year, although he had, now and then, stitches in the left side of the chest and a little cough.

In November, 1947, he was admitted to the Thoracic Surgery Department for observation. X-ray photographs of the lung showed growth of the tumour

EXPLANATION OF PLATES.

FIG. 1.—Case I. Liver with metastases. Upper left corner : tumour from brain.
FIG. 2.—Case I. Brain with tumour in the old operation-cavity.
FIG. 3.—Case I. The intra-cranial tumour found at autopsy (× 95).
FIG. 4.—Case I. Metastasis in the liver (× 95).
FIG. 5.—Case II. The intra-cranial tumour found at autopsy (× 95).
FIG. 6.—Case II. Metastasis in the lung (× 95).



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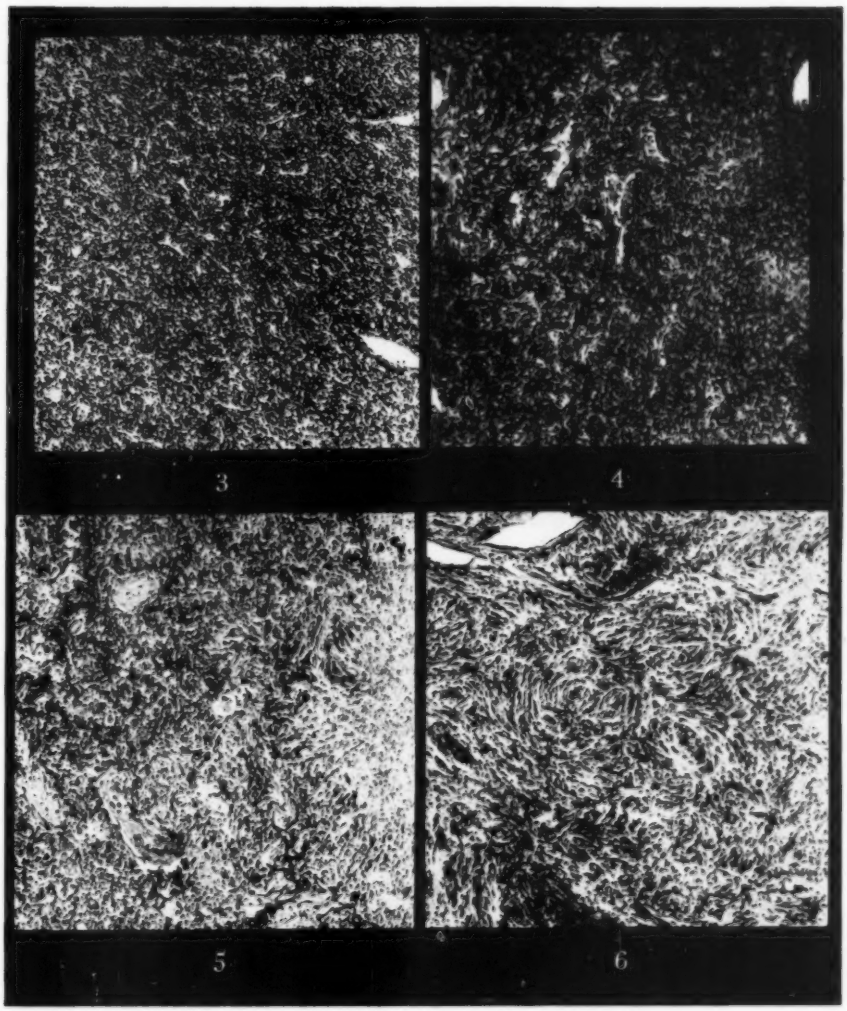

Christensen, Kiær and Winblad.

on the left side. Tomography and bronchoscopy showed the lung to be otherwise normal, and there were no signs of any primary tumour. Consequently it was decided that the left-sided tumour should be extirpated. However, it was realized that it could be a metastasis from the meningioma removed 9 years before, although microscopical examination had shown it to be a benign tumour.

At operation on December 15, 1947, a tumour the size of a walnut was removed from the lower and outer part of the upper lobe of the left lung, where it was adherent to the chest-wall. Microscopical examination during the operation showed it to be a meningioma with psammoma bodies. Then a round, well-demarcated tumour the size of a fist was removed from the mediastinum, intimately connected with the upper lobe. When two tumours were found in the lung pneumonectomy was decided upon and performed without difficulty. The larger tumour, which weighed 350 g., gave the impression of being a mesenchymal tumour of a neurinomatous or fibrous character. The little tumour was harder and more fibrous.

Blood-stained fluid appeared in the left pleural cavity during the first days after operation ; this was removed several times, but on the third day the patient suffered from shock followed by immediate death.

Autopsy.

A tumour was found at the site of the operation of 9 years before. It was tough and fibrous in consistency, the size of an almond, adherent to dura over the left central posterior gyrus towards the mid-line. In the left chest cavity there was a little more than a litre of partly coagulated blood arising from insufficient ligature of an artery.

Peripherally in the right lung a solid tumour was found at the site that was indicated by X-ray examination.

Microscopical examination of both tumours from the left lung removed at the operation showed numerous meningeal cells and a plexiform structure with whorls, which had in many places a collagenous centre and occasional psammoma bodies (Fig. 6). The histological diagnosis was meningioma with pulmonary metastases.

A tumour was also found in the right lung ; it appeared to be a cylindrical epithelial cell carcinoma situated between the alveoli ; no meningioma cells were found here.

Summary of Case II.

A man, 40 years old, had been operated on at the age of 31 years for an intracranial meningioma on the left of the mid-line. He had had right-sided Jacksonian fits for 6 months. With luminal treatment he was nearly well for 8 years, a few jerks in the right leg being all that remained. Eight years after operation the patient showed signs of left-sided lung tumour, which did not improve with X-ray treatment and increased in size during it ; a left pulmonectomy was performed and two tumours were found. The patient died 3 days later from an arterial haemorrhage.

At autopsy a recurrence of the intracranial tumour was found. Microscopical examination of this as well as the two left-sided lung tumours showed the same picture as the specimen from the original operation, which was that of a typical

meningioma with psammoma bodies. (The right-sided lung tumour appeared to be a cylindrical bronchial carcinoma without admixture of meningioma tissue).

DISCUSSION.

On going through the literature we find after 1941 five verified cases of meningeal tumours with extra-cranial metastases. In earlier literature there is much information about similar cases, but the descriptions there are often in completeand the histological diagnosis not verified, as with Klebs' (1889) case, where there were small tumours both in pia and lungs. One gets the impression that both are metastases, and that an unknown primary tumour has been in a third place.

Lindner (1902) reports a case of a man, 63 years old, where a tumour in dura on the base of the cranium and at the same time a tumour in the bladder were found at autopsy, both looking like an adenocarcinoma. His idea is that the meningeal tumour was primary and the one in the bladder secondary. However, the possibility that it was an adenocarcinoma in the prostate gland with an invasion in the bladder and metastasis to dura cannot be overlooked, especially as the shown psammoma bodies may have been amyloid bodies.

Cushing and Eisenhardt (1938) in their extensive work on meningiomas describe a case with extracerebral metastases. The patient was a woman, 35 years old, having been operated upon 19 times in the course of 13 years for a recurrent parasagittal tumour diagnosed as a meningioma. The histological picture was seen to have changed at the last operation to one of an adenomatous character. At autopsy a well-demarcated tumour the size of a nut with the same histological picture was found in the right lung. As the intracranial tumour had altered its character at the last operation and no longer looked like a meningioma, there is a possibility that the intracranial tumour removed at operation was a metastasis from a symptomless carcinoma of the lung. This would fit in more closely with the histological picture, especially so since it is not rare to discover a primary tumour through symptoms due to intracranial metastases.

From Roumania a case of meningioblastoma with abdominal metastases has been published (Derevici, Ionescu and Smilovici, 1937), but the description of the metastases is uncertain. Also Pendergrass and Wilbur (1928) have published a case-history from a patient with a meningioma and metastases to the lungs ; these have, however, only been shown by X-ray examination.

Five cases have been found in the literature in whom extracranial metastases from meningeal tumours have with certainty been detected, and which show, histologically, all transitions from benign, fibroblastic meningiomas to malignant, anaplastic sarcoma (Russell and Sachs, 1942, three cases ; Hamblet, 1944, one case ; Jurow, 1941, one case). These cases are shown in Table I with the two cases described here.

The average age of these seven patients is 35 years ; there are four women and three men. The shortest case-history is of two months' duration, the longest, 24 years. Five of the seven patients have been operated upon, four of them two or three times ; no operation was performed on two of them. The tumours were all unilateral and localized to the convexity of the brain. Six of them were parasagittal, one localized to one of the temporal lobes ; they were capsulated,

TABLE I.

	Age and Sex.	Duration.	Operation.	Localization.	Demarcation.	Metastasis.	Histological diagnosis.
Jurow	72 F.	?	...	Parasagittal	Well demarcated	R. lung	Transitorial meningioma with psammoma bodies.
Russell and Sachs. Case 1	33 F.	22 months	Three times with an interval of 1 and 1½ years	„	Ditto, capsulated	Lung, pleura, lymph nodes, bone	Malignant arachnoid fibroblastoma.
„ „ Case 2	38 F.	15 years	Twice with an interval of 13 years	1. Temporal lobe	Well demarcated	Liver, lung, mediastinum	I. Meningioma. II. Fibrosarcoma.
„ „ Case 3	12 F.	6 „	Three times with an interval of 2 months	Parasagittal	Fairly demarcated	Pleura	Poorly differentiated fibrosarcoma.
Hamblet	42 M.	2 months	...	„	Well demarcated	Liver	
I. Own case, Copenhagen	38 M.	24 years	Twice with an interval of 7 years	„	Ditto	Liver, kidney	Fibrosarcoma
II. Own case, Malmö	40 M.	13 „	Once	„	„	Lung	Meningioma.

and there was a tendency to invade the brain in only one of the cases (Russell and Sachs, 1942, Case No. 3) by a tumour which recurred after operation.

Histology revealed no feature common to all the tumours which might explain the tendency to metastasize.

Among Russell and Sachs' (1942) three cases there is in Case 1 a rather highly differentiated cellular tumour with oval cells lying in some places in streaks or a concentric arrangement with much calcification. Despite this numerous mitoses are seen. The cells form collagen fibrils with reticulin in between. Thus, in this tumour there are many features in common with a meningioma differing from it in that there are numerous mitoses, fibril-forming cells and reticulin, so the diagnosis was malignant arachnoidal fibrosarcoma.

The tumours from Russell and Sachs' (1942) second patient and our patient No. 1 show the same histological picture : a highly cellular tumour with cells similar to fibroblasts lying here and there in winding streaks. The tumour from Russell and Sachs' (1942) patient has many mitoses, but that from our patient has none. In both tumours there are collagen fibrils and reticulin. In both cases meningioma was diagnosed from the specimens obtained at operation, but later it was altered to fibrosarcoma. The case-histories are long, 14 and 22 years respectively, and both of them had metastases in the liver. Russell and Sachs' case also had them in the mediastinum, while in our patient there was a small metastasis in the kidney. Our patient had a local recurrence, but that of Russell and Sachs did not.

The tumour of Russell and Sachs' third patient was, histologically, seen to be very malignant, partially anaplastic, with numerous mitoses, and many of the cells were undifferentiated. In some places the cells were round with plump, reticular, chromatin-rich nuclei, in others the formation was almost the same as in the above-mentioned patients. At operation the tumour was found to be capsulated, but at the autopsy there was invasion into the left frontal lobe and spread of the tumour in large areas of the dura. There were also metastases in the pleura, bladder and right lung.

Jurow's (1941) patient and our second patient show tumours whose formation was that of a typical, transitional meningioma with whorl formations and psammoma bodies. The metastases in the lungs of both the patients show the same picture.

Hamblet (1944) found that his patient also had a transitional meningioma, with whorl formations, which was highly cellular and contained mononucleated giant cells. There were also metastases in the liver showing the same histology.

There is thus a gradual transition from a partially anaplastic tumour to a highly differentiated meningioma in the material referred to in this paper.

CONCLUSION.

The reason for the metastases occurring in these patients is not clear, especially in view of the fact that every year all over the world thousands of meningeal tumours are verified either by operation or autopsy. Only one of the tumours mentioned was locally malignant, but none showed any tendency to invade the vessels, either in our material or among the cases published in the literature.

The operation has not in itself been the cause of the metastasizing of the tumours in all the 7 patients, since 2 of them were not operated upon. Only one of the patients had clinical symptoms of his metastases.

X-ray treatment had no effect in causing the tumours to metastasize because only some of the patients were treated with them.

The contention that all these cases had primary intracranial tumours with extracranial metastases is borne out by the fact that all cases showed symptoms of intracranial tumours for periods up to 22 years, and only one patient (our own case No. II) had symptoms of an extracranial tumour.

SUMMARY.

Two cases are described here in which primary intracranial tumours have metastasized extracranially :

Case I : A 38-year-old male with a history of sensori-motor Jacksonian convulsions had, at the age of 14, a craniotomy with the removal of a tumour on the right spheno-parietal sinus. At the ages of 21 and 37 years he suffered from recurrence of symptoms. At the age of 38 he died in convulsions. The autopsy showed a recurrence of the tumour in the right motor region, several tumours in the liver and a small one in the right kidney. Histological diagnosis : fibrosarcoma with metastases to the liver and right kidney.

Case II : A 40-year-old male had been operated on, at 31 years of age, for removal of a left-sided intracranial meningioma. At 38 years he had symptoms of a left-sided lung tumour, and a pneumonectomy was performed, but he died 3 days later. The lung tumour showed the same histological picture as the intracranial tumour removed 8 years before.

The literature on this subject is briefly reviewed, and the authors find only 5 verified cases of meningeal tumours with extracranial metastases ; these are compared and contrasted with the two cases described here.

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SQUAMOUS DIFFERENTIATION IN MOUSE MAMMAE: SPONTANEOUS AND INDUCED.

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DURING the course of a scrutiny of mammae of old breeding females of several inbred strains of mice in which either very few or no spontaneous mammary tumours had arisen, foci were found in one of the strains of apparently spontaneous squamous differentiation. This differentiation took the form of squamous epitheliosis in ducts and in acini. It was frequently, but not invariably, accompanied by scattered or massed secretory acini in mammary glands that elsewhere were completely involuted. The strain affected was the R III X, a subline of the Paris R III strain, which had previously been freed from the Bittner agent of heritable mammary tumours. A close scrutiny was being made to determine whether mammae of mice free of the agent and of heritable mammary carcinomas were devoid also of adenomas. The result of this first objective is incomplete since it entails survival of a sufficient number of spayed mice to determine the part played by ovarian hormones, but will later be reported. Another experiment in progress during the period of observation included an examination of mammae of young breeding females of the same R III X agent-free subline which had been treated by skin painting with methylcholanthrene with the object of inducing mammary tumours. The experience of many authors indicated that mammary tumours of various sorts would arise (Dobrovolskaia-Zavadskaia and Adamova, 1938; Hval, 1937; Bonser and Orr, 1939; Bonser, 1940; Strong and Williams, 1941; Orr, 1943, 1946; Kirschbaum, Williams and Bittner, 1946; Dmochowski and Orr, 1949). The mice here described had to be killed on account of large skin tumours, despite attempts to avoid them, before any mammary tumours had arisen. Early effects of methylcholanthrene on mammary epithelium were, nevertheless, sought for in accordance with a previous plan to determine the first morphological changes in cells that might be attributed to carcinogenic agents (Pullinger, 1940). It happened that in these young breeders foci of squamous differentiation exactly similar to those in the old untreated females were found but three times as often as in untreated mice and in greater numbers per mammary gland (Table III). In both treated and untreated females atypical squamous proliferation and infiltration were also seen associated with the simple squamous foci. As will be recorded in detail, a few squamous-celled mammary carcinomas occurred in the untreated group and also in mice in a later experiment in which methylcholanthrene was injected in oil. This result conforms with the induction of squamous mammary carcinomas in agent-free breeds by Kirschbaum *et al.* (1946), and by Dmochowski and Orr (1949).

The present paper sets out to describe the morphology and method of detection of these squamous foci, to estimate their incidence, to assess their relationship

to the unmixed squamous carcinomas of the mammary gland and to discuss their origin and significance. The origin of mixed squamous and adenocarcinomas is not included in this discussion since it may be more complex.

METHODS.

Source of mice.

The mice referred to in Tables I–IV were derived from the following strains. The R III X were bred by subsequent litter mating from one R III female and one male of the same litter which had been cross-suckled on a nursing C57 black mother in 1944 (Pullinger, 1947). In 228 breeders of this subline freed from the Bittner agent, the following spontaneous tumours have occurred: one mixed squamous and adenocarcinoma at 23 months old in a breeder spayed at 20 months; one unmixed squamous-celled carcinoma of mammary gland at 22 months in a mouse spayed at 20 months; one unmixed adenocarcinoma of the mammary gland at 19 months old in an intact animal; two sarcomas at 7 and 14 months old respectively; many lymphomas; many pulmonary adenomas and one carcinoma; one papilloma of the skin.

The C57 black females were derived from a pure line bred in these laboratories. No spontaneous mammary tumours have been recorded in this strain. The females actually examined were drawn from an experiment in which they had been cross-suckled on Strain A nursing breeders. These also were being examined for adenomas.

The Simpson mice were not pure line but were inbred old breeders from a stock maintained in these laboratories. All old Simpson females examined had been spayed 4 months before being killed.

The C3H old breeders were control animals derived from an experiment recorded by Vazquez-Lopez (1949).

Management of R III X breeders.

(a) *Spontaneous lesions.*—These had been found at and after the age of 12 months and were subsequently sought for systematically. Old mated females which had borne from none to eight litters were segregated from males in order to allow complete involution in the mammae. No judgment is possible concerning the presence or absence of focal changes if the glands have been functioning previous to death or if involution is far from complete, nor would these foci be found with certainty among the multiple adenomas of the original R III breeders still containing the Bittner agent even though the breeders were spayed. At death or when killed, all 10 mammae of all mice were fixed, stained and examined in bulk according to the method of Gardner, Diddle, Allen and Strong (1934), only slightly modified. All mammae were examined, after clearing, by the author.

(b) *Induced lesions.*—Young females about 4 months old were taken from litters where, as often as possible, a litter mate was available as control to ascertain that the Bittner agent was absent from experimental as well as breeding stock. Both experimental and control animals were separately mated with males also from the same litters when available. Experimental mice were treated twice weekly with methylcholanthrene 0·2 per cent in acetone at sites in rotation on epilated surfaces. In spite of treatment at 5 different sites in rotation very

many skin tumours were induced, making it necessary to kill these mice at an earlier time than mammary tumours could be expected with this technique which, in the event, appeared unsuited to the original purpose. Application to skin in oily solution is evidently very much to be preferred (Orr, 1946). The IF strain used and bred originally by Bonser (1938) may also be more responsive. The total and relative yield of mammary tumours in Orr's experiments was high. Although no mammary tumours grew in the mice under report, all the mammae were as closely examined as those of the untreated breeders.

The control untreated animals of this series were left to live as long as possible since their purpose was to provide proof excluding joint action with the Bittner agent. Actually they failed as a set to breed well or live long and their numbers were made up by the inclusion of four breeding stock females living at the same time (Table III). The mammae of 16 young breeders aged 7–11 months of the ordinary R III X breeding stock were also examined.

In another experiment subsequently undertaken to obtain mammary tumours by chemical carcinogenesis, spayed females of the R III X strain were treated with two measured doses of 400γ each of oestrone and with an injection in olive oil of 8γ of methylcholanthrene. Two squamous-celled mammary carcinomas appeared within 14 weeks. Two simple squamous foci and one squamous plus acinar proliferation were found within the same time. These findings confirmed the results of other authors already referred to of induction of mammary tumours with methylcholanthrene or other carcinogenic chemicals in low cancer strains and also specifically in the absence of the Bittner agent (Kirschbaum *et al.*, 1946; Dmochowski and Orr, 1949). The single spontaneous adenocarcinoma found in 228 breeders occurred in a mouse aged 19 months, and it was not accompanied by any adenomas. The characteristic tumour of the Bittner

EXPLANATION OF PLATES.

FIG. 1 AND 2.—Paired photomicrographs taken with usual light (Fig. 1) and with polarizer (Fig. 2) of a piece of bulk-stained involuted mammary gland showing two lobules of ducts and former acini converted into keratinized epithelium. × 47.

FIG. 3 AND 4.—Paired photomicrographs taken with usual light (Fig. 3) and with polarizer (Fig. 4) of a piece of bulk-stained involuted mammary gland showing a lobule of keratinized squamous cells combined with doubly refracting lipoid. × 47.

FIG. 5.—Section of a mammary duct from an 8-month-old mouse treated with methylcholanthrene showing tongue of keratinized epithelium projecting into the lumen from an origin on the duct wall. × 200.

FIG. 6 AND 7.—Paired photomicrographs taken with usual lighting (Fig. 6) and with polarizer (Fig. 7) of a section of mammary gland from an 8-month-old mouse treated with methylcholanthrene showing portions of ductules and acini converted into keratinized epithelium. Multinucleated plasmodial cells are visible round the honeycomb of the squames. × 47.

FIG. 8.—Section from same mouse as Fig. 6 and 7 showing normal and completely keratinized acini in the same lobule without transition stages. × 200.

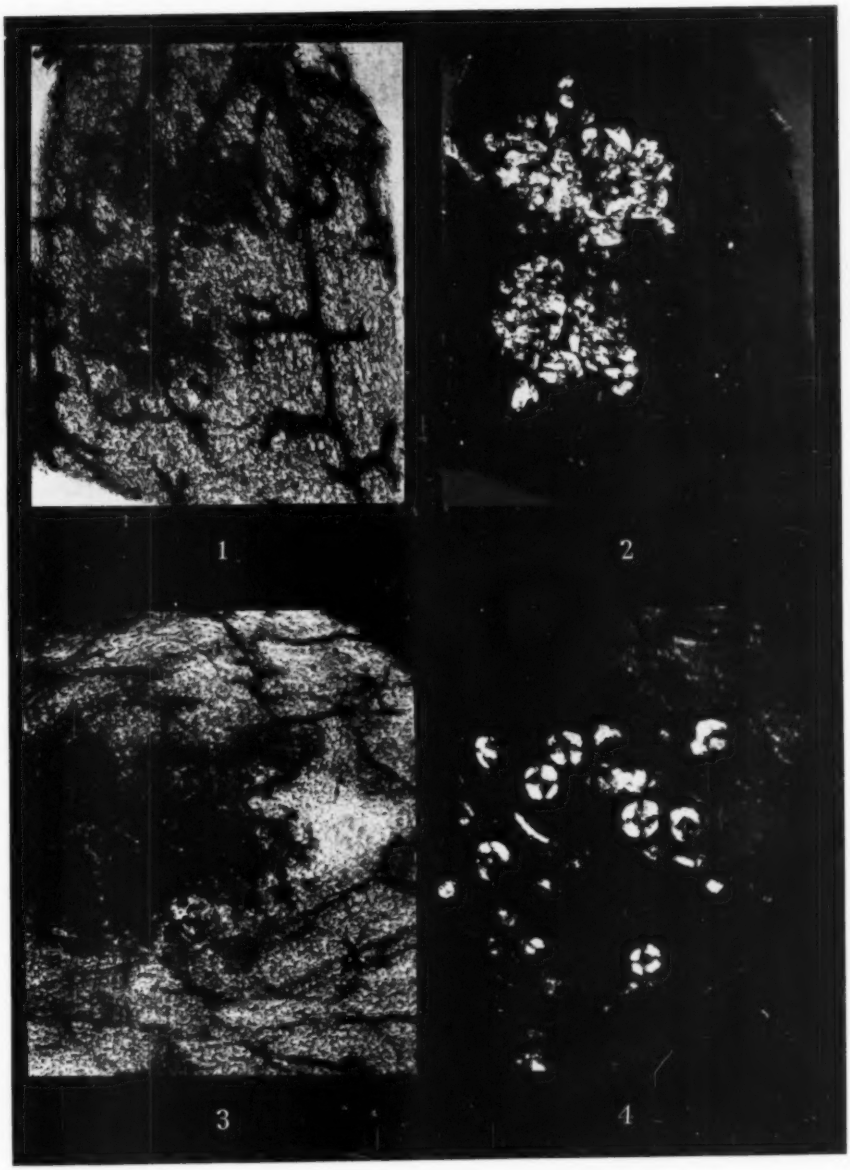
FIG. 9.—High power photomicrograph of curved keratinized ductule in Fig. 6 and 7 showing two multinucleated plasmodial masses, one among keratinized cells, the other at their border but within the basement membrane. × 200.

FIG. 10.—Section of a spontaneous active focus of squamous epitheliosis associated with acinar proliferation and lymphocytic infiltration in a 23-month-old female. × 200.

FIG. 11.—Section of a spontaneous squamous focus at tip of a lobule of acini in a 2-year-old female spayed at 20 months. × 200.

FIG. 12.—Section of a small infiltrating lesion of duct wall in a breeding female treated with methylcholanthrene. × 200.

FIG. 13.—Section of a spontaneous squamous and adenocarcinoma in a 23-month-old female showing recent proliferation of epithelial cells on one side of keratinized laminae and the presumed original focus of squamous differentiation on the other. × 200.



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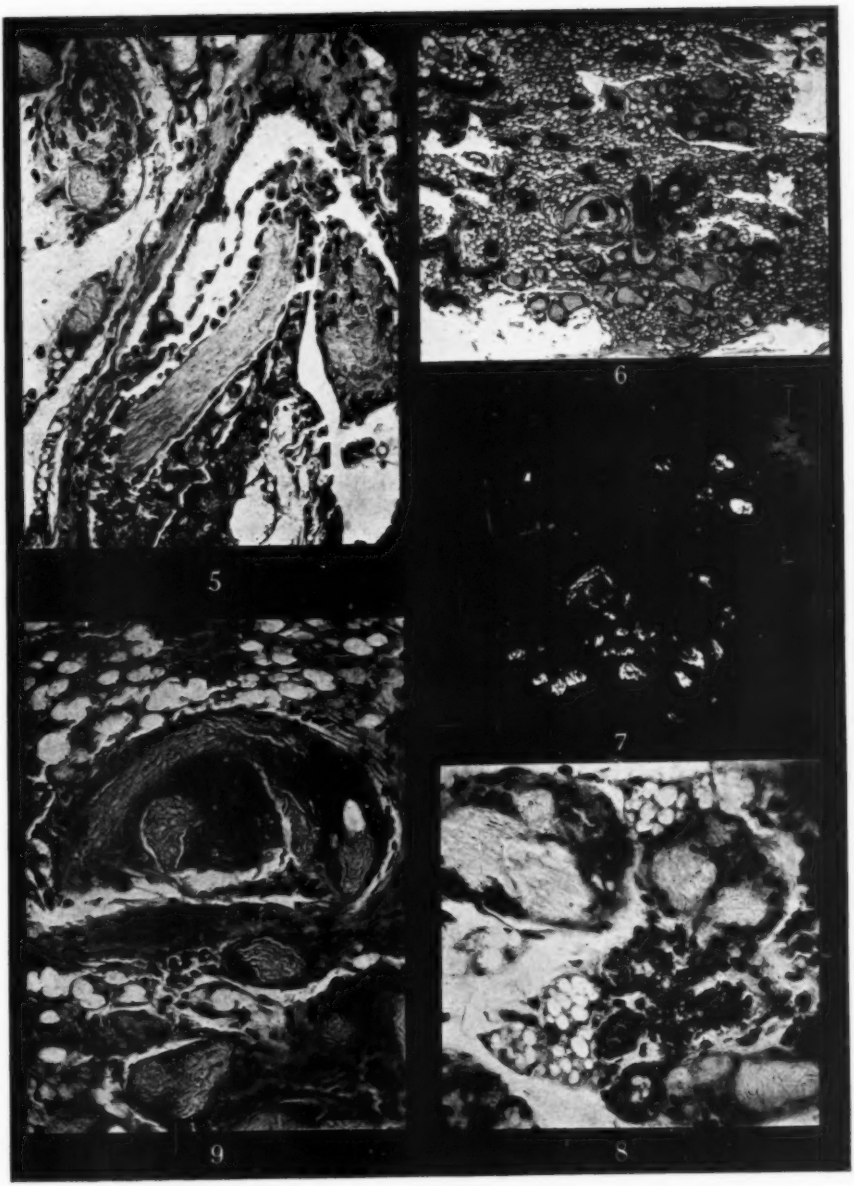

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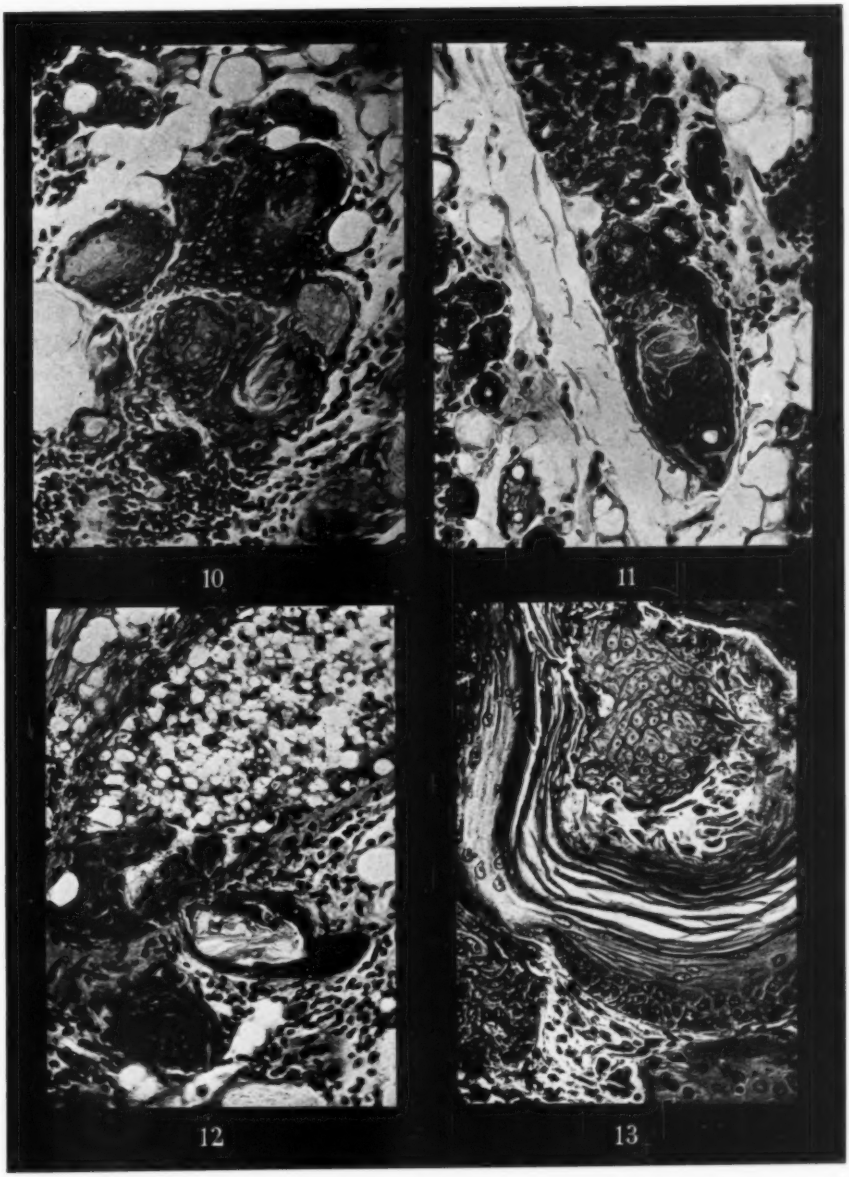

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agent in this strain as in others is an adenocarcinoma associated with multiple adenomas appearing at 8½ months old and onwards. The same experiment showed also the point that may be inferred from Orr's work with male mice (1943), that dosage relationships in respect of tumour induction between oestrogen and carcinogen are amenable to rough estimation.

Detection of squamous foci.

Some squamous foci are detectable prior to fixation. Those on the surface of a gland are visible with a binocular dissecting microscope magnifying 7 to 14 times. They resemble ducts and ductules that have not involuted and appear to contain secretion or concretions, but this is not their usual nature. After fixation their opacity and shape are still visible. When bulk-stained and cleared they tend to disappear and only when accompanied by considerable acinar proliferation, which takes up haematoxylin deeply, is one's attention drawn to them. Thus, if one wishes to detect all of them to estimate incidence, the property of the keratin they contain of polarizing light is best made use of. Paired photos of the same fields made with ordinary lighting and polarized light are shown in Fig. 1, 2, 3, 4, 6 and 7. When such areas are found they can be cut out for separate mounting or for microscopic section. Twenty-four of these foci have been cut in serial section from spontaneous and 27 from induced foci. No differences were found between spontaneous and induced foci.

Microscopic appearances.

Both spontaneous and induced foci may be grouped into (i) inactive, (ii) active and (iii) active with signs of infiltration.

(i) *Inactive foci.*—These consist of focal differentiation of duct epithelium into keratinized tongues (Fig. 5) and conversion of clusters of what appear to have been some or all of the acini of one or more lobules into irregularly globular masses of keratinized squames (Fig. 6, 7, 8). These squames are eosinophil, are connected by intercellular bridges, are stainable by Gram's method and they polarize light. When yellowish alcohol-soluble eosin is used to stain them and the sections are subsequently examined while they are washing in tap water, the squames take up the yellow part of the dye more selectively than anything else in the tissues. The yellow colour tends to wash out on dehydration, but with a little practice some may be retained. This colour, together with the visibility, yet absence of staining capacity of the nuclei, and the polarizing property of the whole, serve to identify these old original foci in the few fully developed squamous carcinomas that occur. The complete lumens of presumed former acini are filled with squames of cells that have previously proliferated, thus meriting the term " epitheliosis." No intermediate stages have been found. Either the acini appear quite normal or they are completely transformed, as in Fig. 8. At the periphery of the squames a few flattened nuclei of indeterminate nature are visible and at one part of it there are almost always to be found multinucleated plasmodial masses within the basement membrane (Fig. 9). Now and then one sees them among the squames (Fig. 9). Since they are invariably situated within the basement membrane and none has ever been seen in surrounding connective tissue, they appear to be of epithelial origin. The appearance as of macrophages attracted to some foreign body, such as keratin might be, was never seen. There is no connective tissue reaction in and around

the inactive foci. Other inactive areas are composed of mixed squamous and acinar proliferations in greatly varying proportions that appear to have been arrested in the state in which one finds them.

(ii) *Active foci.*—Normally stained cytoplasm and nuclei of squamous epithelium and cells in mitosis are found in active foci, so-called because they appear to have been growing when fixed. The arrested squamous change is always present as well. Associated acinar proliferation may have been so active as to hide the squames. These can then only be found in serial sections (Fig. 10, 11). A very small example of an active focus is shown in Fig. 11 because it is a convincing specimen showing an origin from previously differentiated acinar cells. This small focus at the tip of a lobule of acini was traced in serial sections and, although the duct (also undergoing squamous differentiation) approached it, yet no actual connection could be found.

(iii) *Infiltrating lesions.*—Infiltration of tissue outside the basement membrane or walls of ducts (Fig. 12) is an indication of malignant change. It is usually, but not invariably, accompanied by round cell infiltration of the whole focus.

Transition stages.

There is, on the whole, a lack of transition stages either from cubical or columnar to squamous epithelium or from inactive to active foci, or from active to frankly malignant growth. These foci are presumed but not the proven source of the purely squamous-celled mammary carcinomas. There is, however, no other source. Two squamous cysts were found, one completely keratinized, the other with evidence of active growth at the edge.

Incidence of lesions.

The incidence of spontaneous and induced foci is recorded in Tables I to IV together with some data from other sources. In addition, W. U. Gardner (personal communication) allows me to mention that he has found squamous foci in agent-free C3H mice. The frequent induction in IF and C57 black mice of squamous mammary carcinomas by methylcholanthrene suggests that spontaneous squamous differentiation may occur in these mice also (Orr, 1943; Dmochowski and Orr, 1949).

Squamous foci were described by Haaland (1911) in stock mice. From Tables I–IV it will be seen that the overall incidence of spontaneous lesions was

TABLE I.—*Incidence of Spontaneous Foci.*

Strain	Age in months.	Number of mice.		Per cent.	Number of mammae examined.	Largest number of foci seen per mouse.
		Examined.	With foci.			
R III X	12–24	189	39	20·6	1890	5
C57+	12–24	57	0	0	570*	0
C3H	12–24	19	0	0	190	0
Simpson	12–24	20	0	0	200	0
Other authors†	..	..	..	..	..	..
C3H—(or Zb)	Breeders	123	0	0	1230	0
C57	,,	90	0	0	?	0

* Only 93 were examined with polarizing apparatus.

† Kirschbaum *et al.* (1946).

W. U. Gardner (personal communication) has found squamous nodules and tumours in untreated C3H mice in the absence of the Bittner agent. These tumours remained true to type when grafted.

TABLE II.—*Incidence of Induced Foci.*

Strain.	Age in months.	Number of mice. Examined.	Number of mice. With foci.	Per cent.	Number of mammae examined.	Largest number of foci seen per mouse.
R III X (Breeders)	7–9	16*	10	62	160	24
R III X (Virgins)	6	14	4	28	140	2
Other authors†	..	..	..	..	..	..
Zb	—	24	4	16	?	?
C57	—	43	0	0	?	0
NH	—	60	0	0	?	0
NH. D. (F1 Hyb.)	7–10	12	10	83	?	?
(NH. D.)^2 (F2 Hyb.)	7–10	15	12	80	?	?

* Three out of four without foci had no litters. One out of ten with foci had no litters.
† Kirschbaum *et al.* (1946).

TABLE III.—*Comparison of Incidence of Squamous Foci : Spontaneous and Induced.*

Number of breeders.	Number with litter mates.	Age at death in months.	Treated with methyl-cholanthrene.	Squamous foci in.	Per cent.
16	12	7–9	Yes	10	62
16	12	17–24	No	3	18
16	0	7–11	,,	0	0

TABLE IV.—*Results of Search for Squamous Foci in 2280 Involuted Mammae.*

Number of mice. (1).	(2).	Age group in months.	Mice with foci : Intact.	Mice with foci : Spayed.	Per cent.	Squamous carcinomas.	Adeno-carcinomas.	Mixed tumours.
228	..	3–30	30	10	17·5	1	1	1
..	189	12–24	29	10	20·6	1	1	1
..	154	12–24	29	..	18·7	0	1	0
..	35	21–24	..	10	28·7	1	0	1
..	35	21–24	9	..	25·7	0	0	0
..	26	3–11	0	..	0	0	0	0

Column (1) includes all mice examined. In column (2) are groups which have been abstracted from the total in column (1).

20 per cent in R III X females and was raised to 62 per cent by methylcholanthrene. The spontaneous foci were found only at and after the age of 12 months and no more than 5 per gland in any one mouse. Induced lesions appeared at 7–9 months and may have occurred earlier. Twenty-four foci were found in one mouse. They were usually more numerous per mammary gland than those that occurred spontaneously.

Origin of foci.

The exact origin and means by which the cells are transformed into squamous epithelium is in doubt. Since all elements containing mammary epithelium are derived from epidermis the morphological transformation is best described as abnormal differentiation, although some of the appearances, especially that illustrated in Fig. 11, allow the possibility to remain of a reversion of functionally differentiated secretory cells to squamous epithelium. In this particular instance the secretory acini were probably already pathological themselves because they

were present in otherwise completely involuted mammae of a 24-month-old breeding female which had been spayed at 20 months. The appearances illustrated in Fig. 1, 2, 3 and 4 suggest but do not prove that whole lobules of acini have been converted into squamous cells. The previous nature of these converted cells remains in doubt because at involution after lactation the secreting cells are destroyed, then at the next lactation are regenerated from the ends of the ducts. One cannot therefore know whether the basal cells of duct origin had undergone keratinization or whether previously differentiated secreting epithelium had done so. Thus it seems possible that pathological secretory epithelium may revert to squamous (Fig. 11) but that the normal functionally differentiated cells do not.

Significance of squamous foci.

The interest of these foci lies in the stimulus which bring them into existence and their fate. It was seen in Tables I to IV that a spontaneous incidence of foci of 20 per cent was raised by methylcholanthrene to 60 per cent. An embryologist might take the view that an inherited tendency, needing no extraneous influence, exists in the direction of squamous differentiation or that, if a stimulus is needed it may be supplied by some quantitative relationship of ovarian hormones, either excess or absence. The experimental pathologist, aware of the existence of naturally occurring carcinogens and of the suggestion that such substances are amenable to elaboration in animal tissues by comparatively simple chemical transformations (Cook, Haslewood, Hieger, Kennaway and Mayneord, 1937) or in virtue of their lipoid content (Hieger, 1949) might assume these to be the stimuli to squamous differentiation. The hypothetical natural stimuli would reach the mammary gland under the usual conditions of breeding in small amounts, presumably the same for all strains, over a long period of time and produce their effects at a late age in only 20 per cent of mice with this tendency. Artificial addition of a larger amount of a powerful synthetic carcinogen might reduce the time required and increase the incidence. Data available at present are inadequate to decide whether or not any innate tendency to change exists in some strains (though it appears this may be so), whether the 20 per cent in R III X mice is purely spontaneous and whether the differentiation is due to hormone imbalance or to undetected carcinogens. All these problems appear amenable to experimental test.

The fate of the great majority of spontaneous foci appears to be arrest in the the natural process of ageing, ending as keratinized clusters. A proportion proliferate within the life of the mouse and might go further were these animals longer lived. A very few appear to develop into malignant tumours. Though only early stages of atypical proliferation and infiltration have been found and two fully developed malignant tumours, there is one sign in both spontaneous and induced purely squamous mammary carcinomas which does point to the derivation of the malignant tumours from the simple differentiated squamous foci. This is the presence in frank tumours of the curious, yellow-tinted, doubly refracting masses of squamous cells whose nuclei are visible but not stainable as though they had undergone coagulation necrosis (Fig. 13). The tumours are distinct in their origin and morphology from those due to Bittner's agent. This conclusion is in agreement with those of Kirschbaum *et al.* (1946) and Kirschbaum (1949) in respect of mixed squamous and adenocarcinomas.

SUMMARY.

Spontaneous and induced foci of squamous epitheliosis in mouse mammae are described and their incidence estimated in R III X mice free of the Bittner agent.

Spontaneous and induced squamous-celled mammary carcinomas are traced to these foci.

The cause of the squamous differentiation is discussed.

I am greatly indebted to Dr. B. I. Balinsky for helpful discussion of functional differentiation in the mammary gland, and to Mr. E. V. Willmott, Postgraduate Medical School of London, for photomicrographs (Fig. 1, 2, 3, 4, 6, 7).

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SOME EFFECTS OF URETHANE ON THE GROWTH AND MITOSIS OF NORMAL AND MALIGNANT CELLS *IN VITRO*.

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DURING recent years the effect of urethane (ethylcarbamate) on various biological materials has been widely studied. Mitotic inhibition in tissue culture (Geyersbach, 1939; Bucher, 1949; Tennant and Liebow, 1940/41) and rat cornea (Guyer and Claus, 1947), has been observed as well as mitotic degenerations in the Lieberkuehn crypts of the intestine of the mouse, though not in thymus or lymph nodes (Dustin, 1947). Wound healing in rats (Lushbaugh, Green and Storer, 1948) and the growth *in vivo* of the Walker rat carcinoma 256

* Sir Halley Stewart Fellow.

has been retarded (Haddow and Sexton, 1946; Green and Lushbaugh, 1949). Ludford (1936) obtained rather inconsistent results with cultures of carcinoma 63 grown in a medium containing urethane, but found in one culture abnormal metaphases, an effect similar to that observed with colchicine.

The specific effect of urethane in mouse and human leukaemia is well known (Kirschbaum and Lu, 1947; Law, 1947; Paterson, Thomas, Haddow and Watkinson, 1946), but whether the reduction of the white blood cells is due to maturation or mitotic inhibition has not yet been decided.

The appearance of lung adenomata in mice and rats following urethane treatment (Nettleship and Henshaw, 1943; Jaffe, 1947; Orr, 1947) and of highly malignant lymphosarcomata and haemangiomata in mice (Noble and Millar, 1948) has been described. The question whether the production of tumours is due to any direct carcinogenic action of the drug on the bronchial epithelium or whether it is secondary to an unspecific irritation and inflammation is still open.

In view of the often inconsistent though apparently selective effects of the drug, it seemed desirable to study its action on growth and mitosis in various normal and malignant tissues of the mouse *in vitro* in order to find whether it affects specifically any given type of cell, and also to compare its action on normal and malignant cells.

The tissues chosen for the experiments were grouped according to the type of cells they produced, fibroblastic or epithelial. Cells of the fibroblast type derived from frontal bone, spleen, and a sarcoma were used in the first series, and epithelial cells from the pancreas, kidney medulla and adeno-carcinoma 63 in the second series.

MATERIAL AND METHODS.

The cultures were obtained from the spleen, frontal bone, kidney medulla and pancreas of newly born mice, from the C57 sarcoma and the adenocarcinoma 63.

They were grown by the hanging drop technique in a mixture of rat and fowl plasma and chick embryo extract in equal parts. Urethane was added in Ringer solution to the medium of the experimental cultures, the final concentration in the medium being 0·4 per cent; a corresponding amount of Ringer solution was added to the medium of the control cultures. The cultures were then incubated and grown for periods of 2 to 4 days. Preliminary experiments showed the peak of mitosis to occur in the normal untreated cultures on the second day of incubation and usually cultures were fixed at that time. In addition, cultures of pancreas and carcinoma 63 were fixed on the fourth day of incubation. They were fixed in Susa's solution and stained with Mayer's or Ehrlich's haematoxylin and mounted whole.

Mitosis was assessed quantitatively by counting all the mitotic cells present in the zone of growth, and the result was expressed as a percentage inhibition or increase of the control value. Abnormal cell divisions, if present, were also counted and expressed as a percentage of total mitosis. The percentage of abnormal mitotic cells found in the controls was subtracted from that seen in the treated cultures. The counts shown in Table I thus refer to the increase seen in experimental cultures.

Each point represents the average of at least a dozen experimental and control cultures in the case of spleen, bone, C57 sarcoma and kidney medulla. For the experiments dealing with pancreas and carcinoma 63 approximately 50 cultures each have been used.

RESULTS.

Effect of urethane on normal fibroblastic growth in vitro.

A. *Frontal bone.*—Fig. 1 shows a typical untreated culture from the frontal bone of the mouse on the second day of growth. By this time a dense zone of growth consisting of several layers of spindle-shaped cells surrounds the central explant. Mitosis is plentiful (Fig. 3). In cultures treated with urethane the size of the zone of growth is much smaller and less dense while mitosis is reduced to about half of the normal value (Fig. 2 and 4, Table I). The incidence of

TABLE I.—*The Effect of 0·4 per cent of Urethane on Mitosis in Tissue Cultures of Normal and Malignant Cells of the Mouse.*

Cultures obtained from—	Type of cells.	Mitosis as percentage of the control count. Period of growth. 48 hours.	96 hours.	Increase of abnormal mitosis as percentage of total mitosis.
Frontal bone	Fibroblastic	46 ± 8·5	..	1·0 ± 0·7
Spleen	,,	56 ± 4·2	..	5·2 ± 3·3
C57 Sarcoma	,,	172 ± 13·4	..	3·0 ± 3·2
Kidney medulla	Epithelial	41 ± 10·0	..	..
Pancreas	,,	38 ± 5·3	57 ± 4·3	..
Adenocarcinoma 63	,,	273 ± 22·0	430 ± 72·5	8·0 ± 2·3

abnormal mitotic figures is not increased in urethane-treated cultures. The size of the resting cells shows a distinct enlargement in contrast to those seen in untreated cultures.

B. *Spleen.*—These cultures gave a similar result. The zone of growth is reduced and mitosis shows a decrease to 56 per cent of the controls, while abnormal cell divisions are absent. There is also an increase in cell size. A small number of degenerate cells can be observed in bone and spleen cultures after urethane treatment.

Effect of urethane on C57 sarcoma.

This was originally induced by methylcholanthrene and has been grafted through several generations of pure line C57 mice. It consists of spindle cells with a fair amount of stroma. The tumour grows vigorously *in vitro* and liquefies the culture medium strongly. After the first day of incubation a dense zone of growth consisting of spindle-shaped cells can be seen. Besides these spindle cells small round mobile cells wander out from the central explant towards the periphery of the culture ; on the first day of growth they form a halo around the zone of spindle cells and also lie in scattered groups above them (Fig. 5). These wandering cells degenerate quickly and disappear after the second or third day of growth.

Mitosis is plentiful on the first and second day of growth, but decreases from the third day onwards (Fig. 7).

In contrast to cultures of frontal bone and spleen sarcoma cultures treated with urethane show an increase in mitosis of 172 per cent as compared with the untreated controls ; there is no rise in the number of abnormal mitotic figures. The density of the zone of growth appears to be greater after the addition of urethane, although its area is not markedly enlarged. On the other hand, the wandering cells are absent (Fig. 6 and 8). The cell size is slightly increased after treatment with urethane.

An interesting feature is the absence of liquefaction in the experimental cultures which, in consequence, had a longer life span than their controls, which had to be changed every two days. As a rule, cultures derived from mammalian tissue need a transfer every 3 to 5 days.

There was no evidence of cell degeneration due to the urethane.

Effect of urethane on normal epithelial growth.

A. *Kidney medulla.*—In normal cultures on the second day of growth the explant develops a sheet of epithelium of varying density which shows a thickening of its edge. The cells are here packed more tightly and stain more deeply than the rest of the epithelium. Mitotic cells are scattered in the zone of growth, their rate amounting to approximately 3 to 4 per cent of resting cells.

In cultures treated with urethane the size of the sheet is greatly diminished and the peripheral bulge is missing. Mitosis is reduced to 41 per cent of the control value. There is no evidence of abnormal cell divisions, but a small number of degenerate cells can be observed. The cell size is also enlarged as compared with the untreated cultures.

B. *Pancreas.*—On the second day of growth about two-thirds of all cultures obtained from the mouse pancreas form sheets of columnar epithelium arranged

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EXPLANATION OF PLATES.

Frontal bone after two days' growth.

FIG. 1 AND 3.—Peripheral part of control cultures. Note abundance of mitosis in Fig. 3. × 40 and × 350.

FIG. 2 AND 4.—Peripheral parts of similar cultures grown in a medium containing 0·4 per cent urethane. Note reduction of outgrowth and mitosis and increase in cell size. × 40 and × 350.

C57 sarcoma after two days' growth.

FIG. 5 AND 7.—Edge of control cultures showing groups of macrophages and scarcity of mitosis. × 100 and × 385.

FIG. 6 AND 8.—Edge of similar cultures grown with 0·4 per cent urethane. Note mitotic cells and absence of macrophages. × 100 and × 385.

Pancreas epithelium after two days' growth.

FIG. 9 AND 11.—Edge of control cultures. Note mitotic cells in Fig. 11. × 140 and × 350.

FIG. 10 AND 12.—Edge of similar cultures grown with 0·4 per cent urethane. Note scarcity of mitosis and presence of degenerate cells. × 140 and × 350.

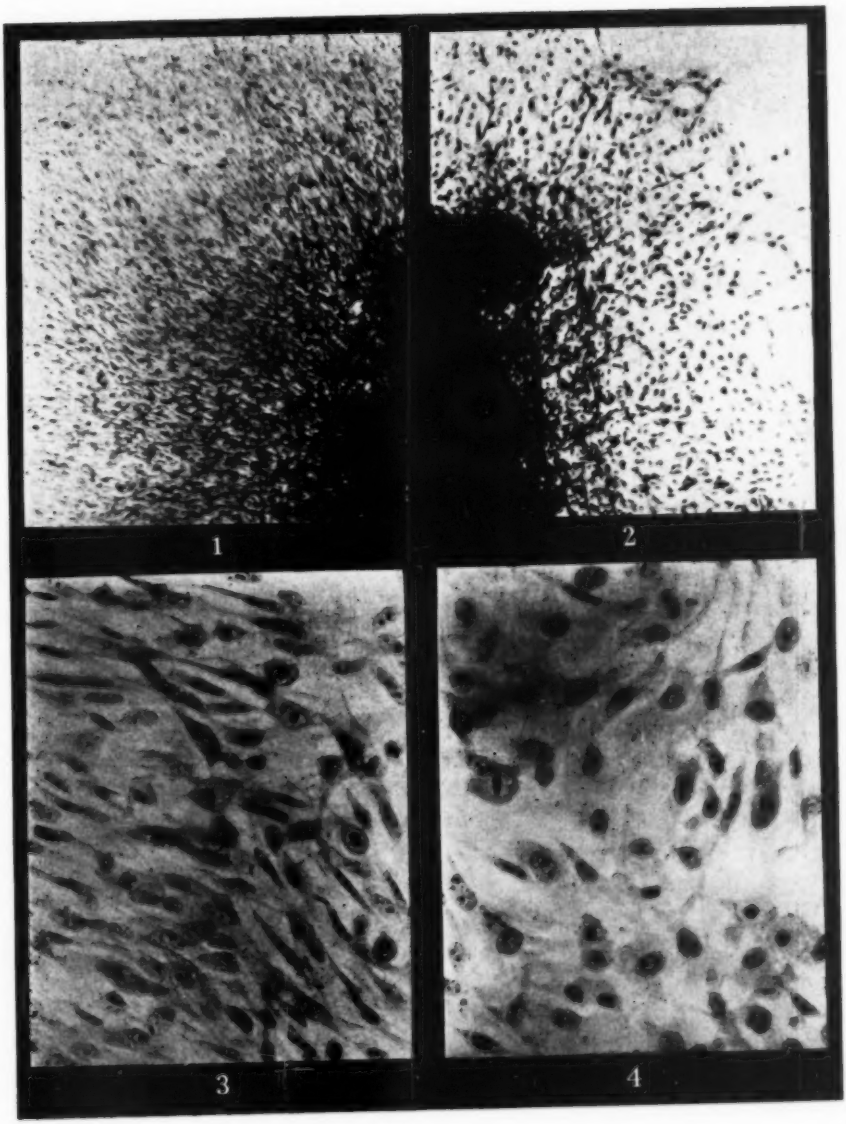
Adenocarcinoma 63 after one day's growth.

FIG. 16.—Control culture. × 60.

FIG. 17.—Culture grown with 0·4 per cent urethane. × 60.

FIG. 18.—Edge of control culture. × 350.

FIG. 19.—Periphery of a similar culture grown with 0·4 per cent urethane. Note abundance of mitosis. × 350.



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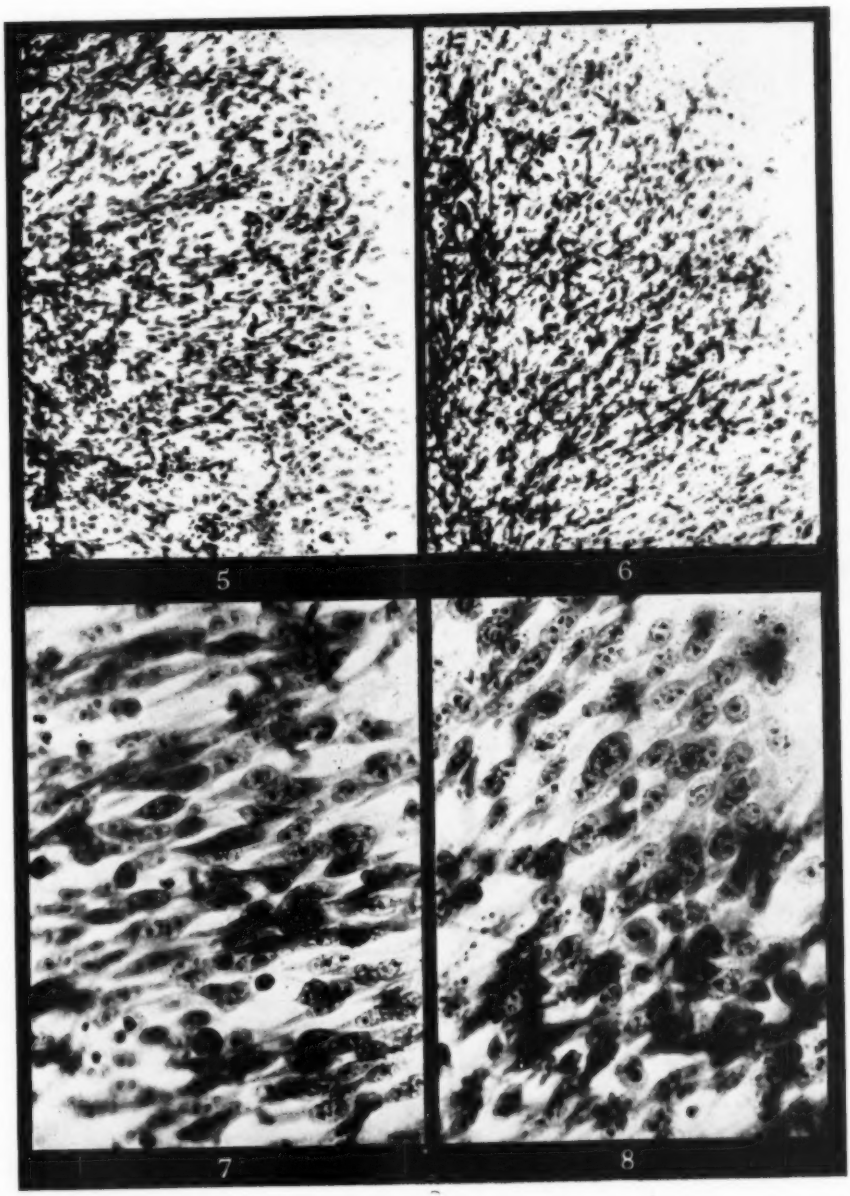

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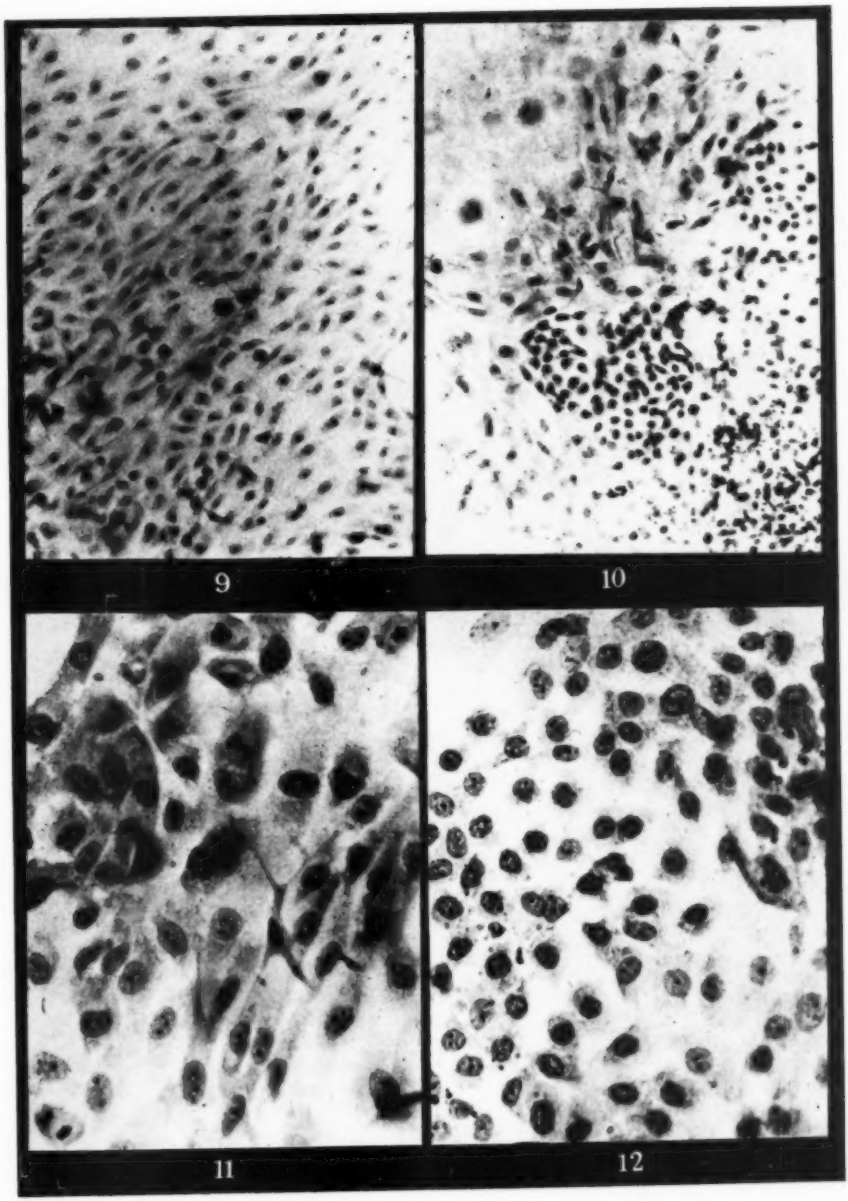

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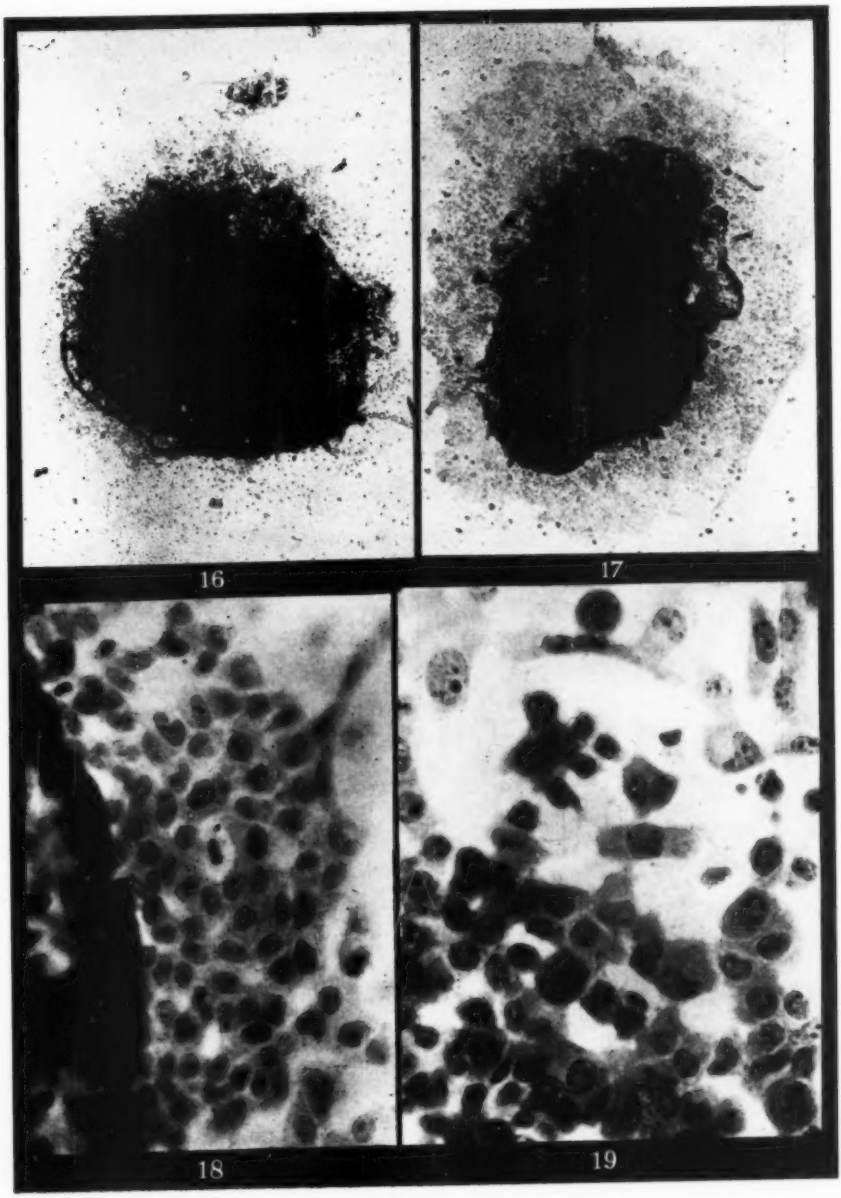

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in a single layer (Fig. 9). A few fibroblasts are, as a rule, present in these cultures. In the remaining third of the cultures fibroblastic growth only is obtained.

Mitotic cells are scattered throughout the outgrowth, and amount to about 4 per cent of the resting cell population (Fig. 11).

The addition of urethane has no effect on the number of cultures forming epithelial sheets. The size of the sheets, however, is much reduced in the treated cultures and only small epithelial islets are produced, while in the controls the explants are entirely surrounded by epithelium (Fig. 10 and 12). At the same time mitosis is reduced to 38 per cent of normal on the second day and to 57 per cent of normal on the fourth day of growth (Table I, Fig. 13). Abnormal mitotic figures were absent, but a small number of pycnotic cells could be seen in the treated cultures.

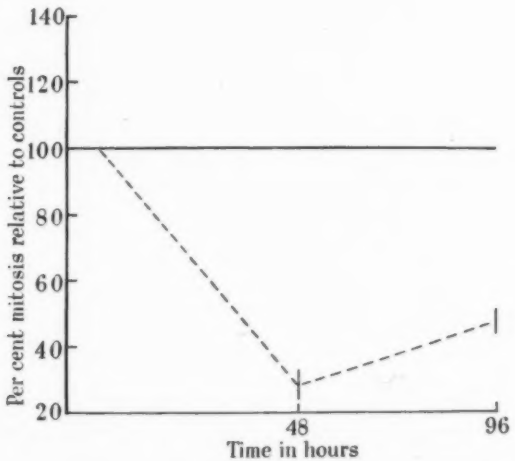

FIG. 13.—Percentage inhibition of mitosis in cultures of mouse pancreas with 0·4 per cent urethane.

Effect of urethane on adenocarcinoma 63.

This adenocarcinoma is a transplantable, rapidly growing, small-celled tumour showing little stroma, and grows readly *in vitro*. After two days' incubation the explant is surrounded by a zone of growth consisting of a sheet of polygonal cells. This sheet is several layers thick near the explant, but it thins out towards the periphery to form a single layer. Mitotic figures are scattered throughout the zone of growth but are more frequent in the neighbourhood of the explant than in the periphery of the outgrowth. Between the first and third day of incubation about 5 per cent of the total cell population are in division at any given moment. Abnormal mitotic figures are seen but as a rule they do not exceed 7 per cent of total mitosis. Some degenerate cells are always present in normal cultures (Fig. 18).

The response to the addition of urethane was a marked stimulation of outgrowth and mitosis.

The appearance of epithelial sheets is greatly accelerated in treated cultures. By the second day nearly all treated cultures show good outgrowth, while fewer and smaller sheets are seen in the controls (Fig. 16 and 17). This difference is

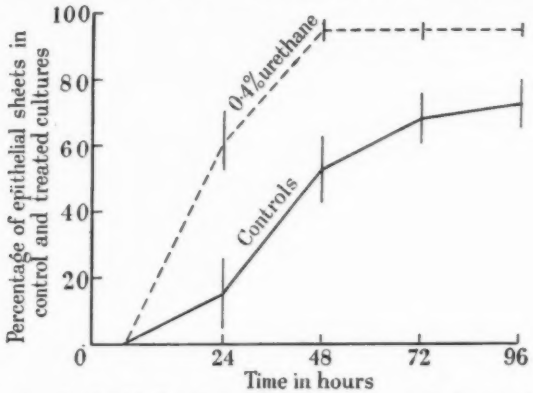

FIG. 14.—The outgrowth of epithelium is speeded up in cultures of adenocarcinoma 63 grown with 0·4 per cent urethane.

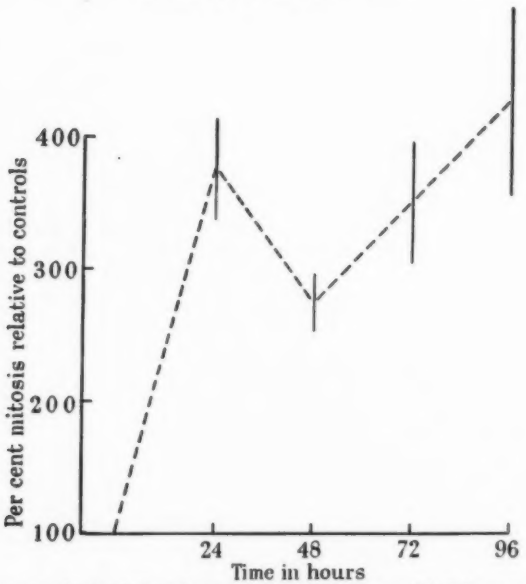

FIG. 15.—Increase of mitosis in adenocarcinoma 63 grown with 0·4 per cent urethane.

greatest on the second day of incubation. Later the controls to some extent catch up with the experimental cultures, and the difference in number and size of epithelial sheets though still evident is smaller on the fourth day of growth (Fig. 14).

A striking rise in the number of cell divisions is observed in experimental cultures and is maintained during the four days of incubation. This rise amounts to 270 per cent on the second and to 430 per cent on the fourth day of growth (Table I, Fig. 15). Fig. 19 illustrates such an increase in mitosis as compared with the control cultures (Fig. 18).

Abnormal mitosis is slightly though significantly increased after urethane and rises to 15 per cent of total mitosis. (Control value : 7 per cent, Table I.) Multipolar cell divisions including a great number of multipolar cells in prophase, polyploidy, divisions of multinucleate cells as well as displacement, aberration and breakage of chromosomes are seen. Multinucleate resting cells probably derived from the multipolar cell divisions are present ; they contain a variable number of macro- and micronuclei, which are sometimes still connected by strands of chromatin.

There is also an enlargement of the cells in the treated cultures and the number of degenerate cells is slightly increased.

DISCUSSION.

The striking stimulation of outgrowth and mitosis in carcinoma 63 and to a lesser degree in C57 sarcoma is in marked contrast to the inhibition of cell division and growth in comparable normal cultures after the same dose of urethane.

The promotion of growth in the carcinoma is, however, accompanied by the production of abnormal mitotic figures, which points to an interference by urethane with the process of division. Similar abnormalities in malignant tissues treated with urethane have been observed in one culture of carcinoma 63 by Ludford (1936) and by Green and Lushbaugh (1949) in the Walker rat carcinoma 256 *in vivo*. The rate of total mitosis in the Walker rat carcinoma was found to be normal, however, and the retardation of growth attributed to the production of non-viable abnormal cell divisions. Haddow and Sexton (1946), on the other hand, believe that the inhibition of tumour growth *in vivo* seen after urethane treatment is due to a differentiation of the tumour cells.

The occurrence of abnormal cell divisions in general does not seem to be specific either for urethane or other narcotics, since similar abnormalities have been observed after various physical and chemical agents, notably ionizing radiations (Lasnitzki, 1943, 1945).

The presence of many multinucleate cells in prophase, on the other hand, seems to be peculiar for the urethane effect. These cells are probably derived from abnormal cell divisions in which the division of the cytoplasm was arrested, although division of chromosomes and reconstruction of daughter nuclei took place in an orderly manner. Such an arrest of cell cleavage has been observed in tissue cultures of rabbit fibroblasts by von Moellendorff (1938) and Bucher (1949) after addition of urethane to the medium.

The number of abnormal mitotic figures found in the carcinoma is not large enough to account for the rise in mitosis by their accumulation, and the normal phase ratio in the treated cultures seems to exclude any prolongation of mitosis in " normally " dividing cells.

The size of the epithelial sheets is greater in the treated carcinoma cultures and they are produced more quickly. This, together with the rise in mitosis,

points to a true stimulation of cell proliferation in the carcinoma and to a lesser extent in the sarcoma.

The mode of action of urethane on cells is not clearly understood. Interference with mitosis has been related to the inhibition of the " activity system " of Fisher and Stern (1942), i.e. the most sensitive part of the overall respiration. Thus Burt (1945) reports the occurrence of abnormal mitotic figures in ciliates when the activity system is differentially suppressed by urethane. Haddow and Sexton (1946), on the other hand, suggest that inhibition of mitosis and growth is caused by interference with the purine metabolism by urethane.

Although interference with respiration or disturbance of purine synthesis might explain the inhibition of mitosis found in the normal tissue cultures, they cannot account for the stimulation observed in the malignant cells.

The stimulation of growth and mitosis in the tumour cells *in vitro* might, at least in part, be due to the surface activity of the urethane (Clark, 1933, pp. 144, 145).

Results obtained some 25 years ago are of great interest in this context. Bauer (1924) reported an increase in the rate of cell divisions of fertilized ascaris eggs kept in a medium containing tributyrine. At the same time experiments on the influence of surface activity on regeneration of epithelium in Triton larvae (Vejnarova, 1924) showed that the regeneration was hastened and mitosis increased in animals kept in a medium containing sodium glycolate. These results lent significance to experiments by Spek (1918) on the relation of surface activity to cell division.

However, if this physical factor were wholly or partly responsible for the promotion of growth in carcinoma 63 and C57 sarcoma, one would have to postulate a special sensitivity of the malignant cells to the lowering of the surface tension of their culture medium. This can probably be excluded since the increase in cell size, no doubt due to the lowering of the surface tension, is found in malignant as well as in normal cells after urethane treatment independent of stimulation or inhibition of mitosis. Stimulation of chick pancreas epithelium has also been reported after the addition of surface active substances to the medium (Katzenstein and Knake, 1931). But it is feasible that in the present experiments any possible stimulation due to the surface activity of the urethane is masked in the case of the normal cells by its antimitotic action.

One might thus assume two factors in urethane responsible for the two different effects seen : a chemical one which inhibits mitosis by a possible interference with respiration or purine synthesis, and a physical one promoting cell division and growth by lowering the surface tension of the culture medium.

The antimitotic effect which reduces mitosis to one-half of the control value in the cultures of normal tissue reveals itself in the carcinoma *only* in the production of abnormal mitosis. The failure of urethane at low concentrations to suppress mitosis and growth in the tumour cells suggests the presence of independent systems of respiration or purine metabolism which are not affected by the drug. However, this explanation must be highly speculative in view of the fact that no consistent differences in the metabolism of tumour and normal cells have been established by modern methods.

The selective inhibition of the wandering cells in the sarcoma cultures is interesting in this context, and indicates that they are non-malignant and derived from the stroma of the tumour.

SUMMARY.

The effect of urethane on growth and mitosis in cultures of normal and malignant tissues of the mouse was studied quantitatively.

The cultures were obtained from the frontal bone, the spleen, the C57 sarcoma, the pancreas, the kidney and the adenocarcinoma 63, and grown for periods of 2 to 4 days in a medium containing 0·4 per cent urethane.

All cultures obtained from the normal tissues showed a reduction of growth and a fall in the number of cell divisions to approximately half of the control value.

In cultures of C57 sarcoma and adenocarcinoma 63 a stimulation of growth and mitosis to 2 to 4 times that of the untreated controls was observed. This promotion of growth is considered a true one.

The carcinoma cultures showed an increase in the number of abnormal cell divisions, particularly of multinucleate cells in prophase.

The difference in effect on normal and malignant cells *in vitro* is discussed. It is suggested that the surface activity of urethane may play a role in the growth stimulation seen in the tumour cells.

I would like to thank Dr. H. B. Fell for her advice and criticism in the preparation of this manuscript, and Mr. G. Lenney for the graphs and microphotographs.

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THE COMPOSITION OF NUCLEIC ACIDS: THE GUANINE AND THYMINE CONTENT OF NUCLEIC ACIDS ISOLATED FROM NORMAL TISSUES AND ANIMAL TUMOURS.

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BECAUSE it is recognized that nucleic acids are macro-molecular in structure it is necessary to examine critically the analytical data upon which the idea of a tetra-nucleotide arrangement is based. Tipson (1945*a*) stated, " On hydrolysing ribosenucleic acid with very dilute ammonia at 115° for 1 hour, or with cold alkali, there is obtained an *equimolecular* mixture of four nucleotides." Also (Tipson, 1945*b*), " Since mild alkaline hydrolysisof ribose nucleic acid gives directly an *equimolecular* mixture of the four mononucleotides, formulas proposed for ribosenucleic acid . . . will be ignored unless they show the union of four mononucleotides."

Evidence has accumulated, however, which suggests that the simple tetra-nucleotide formula of Levene (1921*a*) for deoxy-ribonucleic acids, and similar formulae requiring simple repeating polynucleotide units, do not express accurately the composition of these substances derived from calf thymus or spleen (Chargaff, Vischer, Doniger, Green and Misani, 1949), or for the deoxy-pentose nucleic acid from avian tubercle bacilli or from yeast (Vischer, Zamenhof and Chargaff, 1949). It appears equally probable that the analogous structure applied to pentose nucleic acid may also need revision (Loring, Ordway, Roll and Pierce, 1947).

Gulland (1947) emphasized the fact that four basic factors determine the structure of the polynucleotides: the nature of the individual nucleotides, the nature of the linkages between the nucleotides, the relative positions of the nucleotides and the relative proportions of these nucleotides; and that not one of these factors is known with complete certainty for any one polynucleotide.

In order to provide evidence regarding the composition of such compounds it is necessary to prepare the nucleic acid from various sources, under conditions which are least likely to cause decomposition or degradation, and to determine the units by accurate and specific methods of analysis. If analyses can be made for the constituent units by methods which do not necessitate their complete isolation, errors inherent in such separations would be avoided. The techniques of Hotchkiss (1948) for chromatographic isolation of the purines and pyrimidines from hydrolysed nucleic acids, and also those of Vischer and Chargaff (1948*a*), and Chargaff *et al.* (1949), are notable contributions in this field of work. The latter combined controlled hydrolysis and chromatographic separation of the bases, followed by quantitative estimation of the purines and pyrimidines by ultra-violet absorption spectrometry.

Work has been carried out in this laboratory to obtain data concerning the relative proportions of the purines and pyrimidines in nucleic acids, particularly with respect to the content of thymine and guanine, and results obtained with a

series of nucleic acids, including specimens of deoxyribose nucleic acid from animal tumours, are described in this communication. Values for the nuclear nucleic acids of tumours have not previously been reported.

Absorption colorimetry has been employed for the estimations of these bases, and the methods, therefore, afford data complementary to those given by ultra-violet absorption.

MATERIALS.

Fourteen specimens of nucleic acid were utilized in these studies.

Specimens 1 *and* 2 were commercial yeast ribose nucleic acid and commercial thymus nucleic acid respectively in the form of sodium salts. Samples 1*a* and 2*a* were derived from these by purification in a manner similar to that of Fletcher, Gulland and Jordan (1944) by precipitations from alcohol acidified with acetic acid.

Specimen 3 was prepared in the laboratory from fresh calf thymus according to the method of Hammarsten (1924). Specimen 3*a* was extracted from similar material by the method of Mirsky and Pollister (1946).

Specimens 4 *and* 4*a* were derived from transplanted mouse sarcoma tissue. The tumour was originally induced by intra-muscular injection of 1:2:5:6-dibenzanthracene, and has been transmitted during the course of 12 years through about 270 generations. It is a spindle-celled sarcoma with little stroma. Non-necrotic tumours weighing 4 to 6 g. are obtained after 10 to 14 days in about 80 per cent of the animals inoculated. To extract a specimen of nucleoprotein the tumours from 30 mice were minced in a Waring blendor with 200 ml. of cold physiological saline containing M/1000 sodium arsenate as enzyme inhibitor. The cytoplasmic nucleoprotein is extracted by this procedure and remains in the supernatant liquid when the extract is centrifuged at moderate speed. This was discarded and the operation repeated several times, thus effecting a considerable removal of cytoplasmic protein.

The residue was then extracted for several hours by stirring with M saline when a viscous solution resulted. This was centrifuged to remove insoluble debris, and the nucleoprotein in the supernatant fluid was further purified by repeated precipitations in diluted saline as described by Mirsky and Pollister (1946).

From this nucleoprotein the nucleic acid was separated by the chloroform-octyl alcohol method of Sevag, Lackman and Smollens (1938). The process was repeated four times for Specimen 4*a*.

Fibrous, white preparations were obtained and solutions containing 0·5 per cent were strongly birefringent. Further purification by the method of alcohol precipitation was effected for Specimen 4*a*.

Specimen 5.—Deoxypentose nucleic acid was prepared similarly from transplanted rat sarcomas.

Specimens 6 *and* 6*a* were obtained from livers of untreated rats and from those bearing transplanted sarcomas by extracting the " nuclear " nucleic acid using the above procedure.

Specimen 7 was deoxyribose nucleic acid obtained by a similar procedure from human spleen.

Specimens 8 *and* 9.—These were kindly provided by Dr. F. C. Li, of the Chemistry Department, University of Birmingham. They consisted of a purified

specimen of fibrous fish-sperm nucleic acid, and a specimen derived from the original nucleic acid by procedures used in his studies on the structure of nucleic acid.

METHODS.

Analyses for phosphorus content, deoxypentose, thymine and guanine were carried out on these samples, using the following methods of colorimetric determination in which a Spekker absorptiometer was employed.

Phosphorus estimation.

The method of Holman (1943) was very satisfactory for determining the phosphorus content in hydrolysates obtained by the method of Allen (1940). The blue colour was stable for many hours and replicate analysis gave values differing by not more than 2 per cent for solutions containing between 20 and 100 μg. phosphorus in 2 ml., the final volume for colorimetry being 25 ml. The colour intensity in this range followed Beer's Law very closely when either of the colour filters, Chance OR 2 or Ilford 608, was used.

Nucleic acid value by deoxypentose test.

Comparative values for the different samples were obtained by the diphenylamine test of Dische (1930). This does not yield absolute values for the pentose content, and it seems probable that under the conditions of the test the colours produced by the purine nucleotides are not the same as those obtained by equimolecular amounts of pyrimidine nucleotides. For analyses of nucleic acids a standard interaction time of 15 minutes in boiling water was used and the colours were compared using an Ilford green filter H604. A longer period of reaction with the diphenylamine solution at 100° C. somewhat increases the colour intensity. The colour also increases slowly on standing at room temperature so that accurate timing is necessary. It was found necessary to purify the diphenylamine by twice recrystallizing commercial specimens from petroleum ether. Acetic acid solutions of the reagent thus purified remained colourless over long periods, while " blank " tests gave only a very slight colour.

The chart obtained by plotting " colour produced " against the " quantity of deoxyribose nucleic acid present " in a final volume of 10 ml. of solution was slightly curved between the limits 0·50 and 2·0 mg., but this was reproducible under the standard conditions. The curve obtained with pure deoxyribose under similar conditions (0·10 to 0·50 mg. in final volume of 10 ml.) is similar in type, but the intensity of colour obtained from a known weight of pentose is greater than that produced from an equivalent amount of deoxyribose nucleic acid if this is calculated assuming the conventional tetra-nucleotide structure. This may be because some portion of the deoxyribose in the nucleic acid cannot readily take part in the reaction.

The Dische reaction has been studied by Deriaz, Stacey, Teece and Wiggins (1949). They compared the colour produced by deoxy derivatives and related compounds which give the characteristic blue colour, substances which give no interfering colour including glucose, ascorbic acid and certain aldehydes, those which give green colours—generally not of great intensity, e.g. aldol, glyceraldehyde, and hexals, which give pink violet tints. They concluded that for com-

parative results the presence of such substances as decomposition product during the interaction should not interfere with the test if carried out rigidly. In their experiments they heated for 3·25 minutes in the boiling water bath, in which time the colour development for nucleic acid is incomplete. As shown by experiments described later, for quantitative work it is necessary to hydrolyse for 15 minutes.

Thymine estimation.

The technique described by Woodhouse (1949) was followed in this investigation. Vischer and Chargaff (1948*b*) described a useful micro-scale apparatus for the first stage in the separation of the purines in which the nucleic acid is decomposed with a rapid stream of dry HCl while suspended in methanol. These workers carried out the subsequent step of decomposing the pyrimidine nucleotides in sealed tubes, using concentrated formic acid. In this work the hydrolyses were effected with 30 per cent wt./vol. sulphuric acid. Formic acid has some advantage because it does not decompose cytosine. This, however, was not under examination in the present investigation, while sulphuric acid was known not to interfere with subsequent colorimetry. Sulphuric acid at the temperature (175° C.) employed does not decompose thymine. It can be recovered by the colour reaction, after two hours' treatment with this acid under the conditions employed, without loss.

Guanine estimation.

The method used for estimating this compound colorimetrically was that described by Hitchings (1941). The reaction, which utilizes the Folin-Ciocalteau reagent, although not specific, is very sensitive for guanine. Adenine, hypoxanthine, thymine and cytosine give no colour. Xanthine yields an identical colour value per mol., while 2-oxy-6-amino-purine also gives a similar colour. However, these latter purine compounds were not encountered in the hydrolysates of the samples of nucleic acid which were examined by Vischer and Chargaff (1948) by chromatographic technique and do not appear to be present in nucleic acid.

The method of developing colour with this reagent is simple and speedy, and it can be used accurately over a range of 20 to 150 μg. guanine in a final volume of 10 ml. The colour value follows Beer's Law very closely. The blue colour is very stable, and can be determined in the Spekker Absorptiometer without a filter.

It was necessary to ascertain whether any substances present in the hydrolysis products of nucleic acid would interfere with the accurate analysis of the guanine. The following tests were made during the course of various experiments undertaken to investigate the conditions necessary for the determination of the guanine content of nucleic acid and nucleotides.

The figures given in these and subsequent analyses are means of at least two duplicate experiments made on two initial samples.

A. *Recovery from mixed purines.*—From a solution containing 0·100 mg. thymine, 0·100 mg. guanine and 0·200 mg. adenine, the purines could be precipitated with silver acetate and the guanine determined quantitatively by the Hitchings' colorimetric procedure. From the remaining fluid the pyrimidine-

silver salt could be precipitated by making alkaline with barium hydroxide, and by proceeding with the method for thymine (Woodhouse, 1949) a quantitative recovery as measured by the colour value for this component obtained.

B. *Recovery from guanylic acid.*—The guanine could be determined quantitatively in a solution of a pure sample of guanylic acid after hydrolysing with 5N sulphuric acid.

10 mg. guanylic acid were dissolved in 25 ml. distilled water.

(1) 5 ml. were refluxed with 5 ml. 10N sulphuric acid for 2 hours and then made up to 10 ml.; 1·0 ml. of this solution taken for colorimetric determination.

Found (in original 10 mg. guanylic acid) = 4·05 mg.

Theoretical $\left(\frac{151}{382} \times 10 \text{ mg.}\right)$ = 4·0 mg.

(2) 5 ml. of the hydrolysed solution were nearly neutralized with sodium carbonate, silver acetate was added and the silver purine centrifuged, decomposed with 2 ml. N hydrochloric acid and made up to 10 ml. 2·0 ml. of this solution were taken for the estimation and made up to 10 ml.

Found (for original 10 mg. guanylic acid) = 4·04 mg.

C. *Control tests.*

(1) 10 mg. adenylic acid after hydrolysis gave by direct application of the F.C. reagent a colour equal to only 1·4 μg. of guanine. Thus the hydrolysis products, e.g. adenine or products from ribose, gave a negligible colour value.

(2) 2 ml. pure deoxyribose (provided by Professor Stacey, Chemistry Department, University of Birmingham) containing 1·6 mg. pentose gave a negligible colour with the F.C. reagent, the colour value being equal to 11 μg.

(3) 2·0 mg. guanylic acid contained in 5 ml. dilute HCl were hydrolysed with the Formic acid-HCl mixture (N HCl and 10 N HCOOH) of Graff and Maculla (1935) for 2 hours under reflux. 5 ml. of the mixture were made up to 25 ml., of which 2 ml. were taken for test.

Guanine found . . . . = 61·0 μg.

Theoretical value of guanine in this = 63·6 μg.

(4) No colour was produced with the F.C. reagent by 10 times the amount of formic acid employed in the above test.

(5) A solution containing 1·0 mg. guanine in 20 ml. was refluxed for 2 hours with the concentration of formic HCl acid used in these tests. When aliquots of the diluted solution were tested they yielded theoretical colour, proving that no destruction of guanine took place.

RESULTS.

The values for the phosphorus, thymine and guanine content of the specimens and their colour production by the diphenylamine test compared with commercial thymus nucleic acid are given in Table I. The spleen nucleic acid gave a low value for phosphorus and guanine. This may be due possibly to autolytic changes in the spleen which was obtained post mortem. The nucleic acid was

TABLE I.—*Results.*

	Phosphorus content (%).	Dische value.	Thymine content (%).	Guanine content (%).	Adjusted thymine (%).	Adjusted guanine (%).	Guanine thymine molar ratio.
1. Commercial yeast nucleic acid sodium salt	7·4	—	—	10·4	—	—	—
1*a*. Ditto purified	7·8	—	—	10·0	—	—	—
2. Commercial calf thymus	7·3	100	6·4	8·1	8·0	10·2	0·96
2*a*. Ditto purified (free acid)	8·3	103	7·0	8·4	8·4	10·7	0·95
3. Calf thymus nucleic acid sodium salt (Hammarsten)	7·6	115	7·0	8·0	8·5	9·8	0·875
3*a*. Ditto (Mirsky)	8·4	125	9·0	8·4	9·9	9·2	0·80
4. Mouse sarcoma nucleic acid sodium salt	7·8	109	9·0	13·0	10·7	15·4	1·08
4*a*. Ditto	8·3	114	9·0	13·8	10·0	15·2	1·14
5. Rat sarcoma nucleic acid sodium salt	8·4	127	8·9	14·7	9·8	16·1	1·23
6. Nucleic acid from livers of tumour-bearing rats	7·5	81	7·8	10·1	9·7	12·6	0·98
6*a*. Nucleic acid from normal rat liver	7·4	77	7·4	9·6	9·2	12·0	0·98
7. Nucleic acid from human spleen	6·5	102	5·3	9·1	7·5	12·9	1·30
8. Nucleic acid from fish sperm	8·75	121	7·75	14·4	7·8	14·6	1·46
9. Nucleic acid A	9·8	138	7·8	14·8	7·9	15·0	1·43
"Theoretical" ribose nucleic acid	9·5	—	—	11·5	—	—	—
"Theoretical" ribose nucleic acid tetra sodium salt	8·9	—	—	10·8	—	—	—
"Theoretical" deoxyribose nucleic acid	9·9	—	9·1	12·0	—	—	1·0
"Theoretical" deoxyribose nucleic acid tetra sodium salt	9·25	—	8·5	11·3	—	—	1·0

found to contain a small amount of protein impurity, about 3 per cent, but this could not account entirely for the low phosphorus value. The phosphorus content of the other tissue nucleic acid specimens varied from 7·3 to 8·4 per cent, while the sample of sperm nucleic acid had 8·75 per cent phosphorus and the nucleic acid A practically the theoretical value of 9·8 per cent.

The thymine content was low in the commercial specimens and highest in the sarcoma nucleic acids, while the guanine values were particularly high in the tumour-derived specimens. The values for these units have also been recalculated to ascertain the percentage which would be present if the phosphorus content of the various specimens had been that of the conventional tetranucleotide, i.e. 9·5 per cent for ribose nucleic acid, 9·8 per cent for the deoxyribose nucleic acid and 9·25 per cent for the tetra sodium salt. These values are given in columns 5 and 6 in Table I. The theoretical content of thymine and guanine for a "pure" tetranucleotide nucleic acid are also given. Whereas the thymine values found in our specimens are fairly similar to the "theoretical" figure, the guanine contents of the tumour nucleic acid and the sperm nucleic acid are 25 per cent to 50 per cent above the "theoretical" amount.

It will be observed that on this basis the thymine content varies from 7·8 per cent to 12·0 per cent and the guanine from 9·2 per cent to 16·1 per cent. The high values for guanine in the sarcoma-derived specimens may be of special note.

DISCUSSION.

In appraising the significance of the analytical results the " purity " of the preparations is a major consideration. It is very difficult to assess the quality of a macro-molecular substance or fraction whose structure and composition are under review, since none of the contained elements or units can be accepted as a suitable criterion.

Commercial preparations, and those isolated by methods involving treatment with reactive chemicals, may contain degraded types and decomposition products. Many of the samples prepared for the present investigation were isolated by techniques which are believed to minimize such decomposition ; in some instances an enzyme inhibitor in the form of M/1000 sodium arsenate was added to the initial stages of the isolation of the nucleoprotein with the object of preventing cleavage by nucleo-phosphatases (Klein, 1933) or by depolymerases (Stern, Goldstein, Wagman and Schryvers, 1947).

The complete removal of the protein from the nucleic acid requires special attention, particularly in preparations made from liver and from spleen, and experience showed that the process of dissociation by the chloroform-octyl alcohol method of Sevag, Lackman and Smollens (1938) must be repeated many times to remove the protein completely. Khouvine and Gregoire (1944) recommended as many as 30 treatments.

Phosphorus content.

Even with these precautions the phosphorus content appears to fall short of that required by the conventional formulation of nucleic acid ; the values range from 7·6 per cent. to 8·4 per cent. (the value for nucleic acid A was exceptional, and may be due to the method of preparation), whereas the required figure is 8·9 per cent for the ribose tetra sodium salt and 9·2 per cent for the tetra sodium salt of deoxyribose nucleic acid.

This " low " phosphorus content has also been characteristic of the preparations described by many other workers, e.g. Hammarsten (1924) 7·09 per cent., Jorpes (1934) 5·7 per cent for the barium salt (theoretical 7 per cent), Loring *et al.* (1947) 8·27 per cent to 8·77 per cent for commercial yeast nucleic acids, 8·2 per cent for tobacco virus nucleic acid and 7·78 per cent to 8·3 per cent for certain fractions of yeast nucleic acid. Also the yeast ribose nucleic acid analysed by Vischer and Chargaff (1948*b*), which was purified from commercial material in a similar manner to that used for Specimens 1 and 2 in this paper, had 8·0 per cent phosphorus, while the material they obtained from pig pancreas had 7·9 per cent. The deoxyribose nucleic acid from calf thymus obtained by Chargaff *et al.* (1949) using the Hamarsten (1924) method contained 8·0 per cent, while a similar content was present in a specimen purified via the lanthanum salt and converted to the potassium salt which was used as a standard for the determinations of the deoxypentose content of other specimens. Specimens isolated by these workers by the method of Gulland, Jordan and Threlfall (1947) contained only 7·6 per cent. Beef spleen deoxypentose nucleic acids isolated by the Mirsky and Pollister (1946) process, in which the protein was removed by 9 treatments of a solution of nucleoprotein in physiological saline with chloroform-octyl alcohol, appeared to be of high purity, as measured by the phosphorus content, which was 8·6 to 8·8 per cent. On the other hand, from avian tubercle bacilli a nucleic

acid was isolated, which, after 5 treatments with chloroform-octyl alcohol mixture each of 16 hours' duration, contained only 4·8 per cent phosphorus. By the action of deoxycholate on the nucleo-protein, and subsequent chloroform-octyl alcohol treatment and other purification steps, a specimen containing 6·3 per cent phosphorus was obtained. By electrophoresis a component with 7·6 per cent phosphorus was produced from a crude nucleic acid extracted from ether-washed bacilli with deoxycholic acid.

The above figures indicate the great difficulty in removing contaminating substances from undegraded nucleic acids, but it is possible that some of the variation may be actual and not due to " impurity."

The content of purines and pyrimidines.

Some early reports suggested that the amounts of those compounds obtained by hydrolysis of nucleic acid were not consistent with the tetra-nucleotide theory (Levene, 1921*b*; Steudel, 1935).

Mirsky (1943), however, described the ratio of total purines to pyrimidine in deoxy-pentose nucleic acid as " equimolecular " in specimens obtained from animal tissues and from wheat germ. This conclusion was based partly on experimental evidence, which suggested that the ratio of purines to pyrimidines in nucleic acids could be calculated by comparing the intensity of colour obtained by the Dische diphenylamine reaction (Dische, 1930) with the total nucleic acid as indicated by the phosphorus content. It was believed that the blue diphenylamine colour was produced almost entirely by the purine pentose, and that the contribution of the pyrimidine nucleotide was negligible.

Experiments have been carried out in this laboratory comparing the colours produced by deoxyribose and deoxyribose nucleic acid with the Dische reagent when the standard reaction time of 15 minutes (Siebert, 1940) was used. Also curves were drawn comparing the colour produced after various times of hydrolysis from 5 to 40 minutes, by the isolated sugar and by nucleic acid. The colour development was rapid during the first 15 minutes, and continued at a slower rate for at least 40 minutes. The form of the curves obtained by plotting time against colour for the deoxypentose was the same as that for the nucleic acid. However, on calculating the total amount of pentose which would be contained in a given weight of nucleic acid (assuming a tetranucleotide of conventional structure), the amount of colour obtained in these tests was less than that produced by the equivalent amount of free deoxyribose, but greater than would be produced if the colour were contributed by the purine-combined portion only. It was concluded that the proportions of purine and pyrimidine nucleotides could not be accurately calculated by means of this reaction.

Gulland, Jordan and Taylor (1947) obtained values by electrometric titrations of a specimen of calf thymus deoxypentose nucleic acid sodium salt of high grade (phosphorus, 9·33 per cent; nitrogen, 15·3 per cent; sodium, 6·9 per cent), which indicated that the proportions were 1 radical of guanine, 1 of thymine, 1·2 cytosine and 0·8 adenine. Also, when analyses were conducted on the purine and pyridmidine fractions obtained from the hydrolysate of a sample of yeast nucleic acid by precipitations with silver acetate, results were obtained suggesting that the ratio of purine to pyrimidine nitrogen was 1·86 instead of the theoretical 2·0 for a statistical tetra nucleotide.

More recently Chargaff and his co-workers (1949) have analysed a variety of pentose and deoxypentose nucleic acids and, in some instances, wide divergencies from the equimolecular ratio were found. These are illustrated in Table II.

TABLE II.—*Molar Proportions (M.P.) and Percentages of Purines and Pyrimidines in Nucleic Acids.*

Abstracted from the communications of Vischer and Chargaff (1948*b*), Chargaff, Vischer, Doniger, Green and Misani (1949), and Vischer, Zamenhof and Chargaff (1949).

	Adenine.	Guanine.	Cytosine.	Uracil.	Thymine.	Guanine. / Thymine
Yeast ribonucleic acid :						
M.P.	3·2	3·1	3·0	1·0	—	—
Per cent	9·1	10·0	7·0	2·4	—	—
Pancreas pentose nucleic acid :						
M.P.	3·6	8·8	4·5	1·0	—	—
Per cent	5·7	15·5	5·8	1·3	—	—
Deoxypentose nucleic acid of calf thymus :						
M.P.	1·6–1·7	1·2–1·3	1·0	—	1·3–1·6	0·75–1·0
Per cent	9·2–10·0	7·4–8·8	4·7–4·9	—	7·3–8·4	—
Deoxypentose nucleic acid of beef spleen :						
M.P.	1·5–1·6	1·2–1·3	1·0	—	1·4–1·5	0·8–0·86
Per cent	9·4–9·7	8·5–8·6	4·8–5·3	—	8·4–8·5	—
Deoxypentose nucleic acid of avian tubercle bacilli :						
M.P.	1·1	2·6	2·4	—	1·0	2·6
Per cent	3·9	10·1	6·8	—	3·2	—
Deoxypentose nucleic acid of yeast :						
M.P.	1·8	1·0	1·0	—	1·9	0·53
Per cent	8·5	5·5	3·9	—	8·3	—

It will be seen that the values in the thymus nucleic acid differ considerably from those of Gulland (1947). The purity of the preparation employed by him seems very satisfactory as judged by the values for phosphorus, sodium and nitrogen.

In certain instances, when material is comparable, the values for Chargaff's (1949) analyses have also been calculated on the phosphorus content for a " theoretical tetra nucleotide."

On this basis, the values for thymine in his thymus nucleic acid specimens range from 7·8 per cent to 8·9 per cent compared with our values, 7·8 per cent in Specimen 2*a*, 8·4 per cent in Specimen 3 and 9·0 per cent in Specimen 3*a*. The values for guanine range from 8·0 per cent to 9·6 per cent in his samples compared with 9·3 per cent for Specimen 2*a*, 9·7 per cent for Specimen 3 and 9·2 per cent for Specimen 3*a*. Those specimens which were derived from " normal " tissue cells are therefore fairly comparable in thymine and guanine content. The high guanine values of the neoplastic and sperm-cell products are particularly evident. One specimen of pancreas pentose nucleic acid analysed by Vischer and Chargaff (1948*b*) was also characterized by a high guanine content, viz. 15·4 per cent for a specimen with phosphorus 7·9 per cent or 18·5 per cent calculated to a phosphorus content of 9·5 per cent.

The above figures show that "nucleic acids" isolated by relatively mild treatment from various tissues may have different contents of purine and pyrimidine bases. Before conclusions can be drawn as to the significance of these variations, it will be necessary to examine material and cells of different types of neoplastic and "normal" tissue and organisms, and to extend the analysis to adenine and cytosine. Some further work is now being undertaken in this direction.

SUMMARY.

Specimens of nucleic acid, including those obtained from tumour cells, have been analysed for their content of thymine and guanine.

The guanine content of the fibrous deoxynucleic acid from mouse and rat sarcoma tissue and from fish sperm was greater than that of calf thymus or rat liver nuclear nucleic acid.

The figures provide further evidence that the amounts of thymine and guanine in nucleic acids vary according to their origin and are not necessarily equi-molar.

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CHEMICALLY INDUCED BREAST TUMOURS AND THE MAMMARY TUMOUR AGENT.

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KIRSCHBAUM and Bittner (1945) considered squamous metaplasia as a characteristic change, specific to chemically-induced breast cancer, and pointed out that the different histological appearance of methylcholanthrene-induced and spontaneous breast tumours may be the expression of a different origin of these two types of breast cancer. They found, however, a higher incidence and an earlier appearance of methylcholanthrene-induced breast cancer in mice of a strain known to possess the mammary tumour agent (milk factor) than in mice of the same strain deprived of it. This observation suggested a relationship between spontaneous and induced mammary tumours, namely, that the mammary tumour agent may take part in the origin of both types of tumours. Although their observations indicated a certain strain specificity to the development of methylcholanthrene-induced mammary tumours, the relationship between susceptibility to the development of spontaneous and methylcholanthrene-induced breast cancer remained uncertain. In another series of experiments, Kirschbaum, Williams and Bittner (1946) came to the conclusion that the histogenesis of breast tumours induced with methylcholanthrene is the same either in the presence or in the absence of the mammary tumour agent and differs from that of spontaneous breast cancer in mice of the same strain. In spite of the previously observed difference between the incidence of methylcholanthrene-induced breast cancer in mice with the mammary tumour agent and in mice of the same strain deprived of the agent, Kirschbaum, Williams and Bittner (1946) were inclined to postulate a different aetiology for spontaneous and induced breast cancer because of the observed differences in the histogenesis of these two types of tumours. Strong (1945) considered that the action of methylcholanthrene may either involve a reversal of genetic resistance to the development of breast tumours or lead to the manifestation of a new type of susceptibility different from that involved in spontaneous breast tumours. Thus it was not clear whether the origin of breast tumours induced by methylcholanthrene was the same as or different from that of spontaneous mammary cancer, and the part played by the mammary tumour agent in the development of chemically-induced tumours required clarification. The possibility that the action of methylcholanthrene may result in the appearance of the mammary tumour agent in mice of strains known to lack the agent also required investigation.

In connection with the work quoted and the results described in a previous paper (Dmochowski and Orr, 1949) experiments were carried out in order to find out if the milk factor plays a part in the development of mammary tumours induced with methylcholanthrene.

MATERIAL AND METHODS.

Breast tumours of C3H high- and C57 low-breast-cancer strain male mice induced by combined application of oestrone and methylcholanthrene, of C3H males following treatment with oestrone, and of C57 females after treatment with methylcholanthrene were investigated for the presence of the mammary tumour agent. In addition, breast tumours induced in IF low-breast-cancer strain mice with methylcholanthrene (Orr, 1946) were also examined.

Breast tumour tissue desiccated and stored for periods of time varying from 1 week to 7 months was used. The tumour tissue, immediately before use, was resuspended in distilled water in a proportion of 1:10 and injected subcutaneously every other day into 4–6 weeks old susceptible C57 × RIII hybrid female mice. All females were forcibly bred by removing the first three litters and then allowed to breed in a normal way.

EXPERIMENTAL PROCEDURE AND RESULTS.

Experiment 1.

Four breast tumours induced in IF low-breast-cancer strain females with methylcholanthrene and one breast tumour induced in a C3H male with oestrone and methylcholanthrene were tested for the presence of the mammary tumour agent.

Thirty-six C57 × RIII hybrid females which were litter mates were divided into 5 groups.

The first group of C57 × RIII hybrid females received 5 injections, each of 0·5 c.c. (0·05 g. fresh tissue) of a distilled water suspension of dried IF tumour No. 36.23, giving a total equivalent to 0·25 g. of fresh tissue.

The second and third groups of C57 × RIII females were given 7 similar injections of IF tumours Nos. 35.94 and 37.39 respectively, corresponding to 0·35 g. of fresh tissue in each case.

The fourth group of mice, 12 females in all, received 12 subcutaneous injections of IF tumour No. 34.63, a total of 0·6 g. of fresh tissue.

The fifth group of mice was given 8 subcutaneous injections of 0·5 c.c. distilled water suspension of C3H No. 30 breast tumour induced in a male mouse with combined treatment of oestrone and methylcholanthrene (Dmochowski and Orr, 1949) comprising a total of 0·4 g. of fresh tumour tissue.

As can be seen from Table I, no breast tumours developed in any of the C57 × RIII susceptible hybrid mice given repeated injections of distilled water suspensions of dried breast tumours induced in IF low-breast-cancer strain mice with methylcholanthrene. During this time 4 out of 6 C57 × RIII hybrids, given breast tumour tissue induced in a C3H high-breast-cancer strain male mouse with oestrone and methylcholanthrene, developed breast tumours. All tumours were examined microscopically and were found to be adenocarcinomata. It can be concluded that the mammary tumour agent is present in tumours induced in C3H strain males by combined application of oestrone and methylcholanthrene. Tumours induced in IF low-breast-cancer strain female mice do not seem to harbour the agent.

Experiment 2.

Six mammary tumours, 2 induced in C3H strain males by painting with oestrone, 2 which developed in C3H strain males after treatment with oestrone

TABLE I.—*Administration of Desiccated Induced Breast Tumour Tissues to C57 × RIII Susceptible Hybrid Female Mice.*

Experiment 1.

No. of mice.	Type of dried tumour tissue M (methylcholanthrene-induced) Oe–M (oestrone and methylcholanthrene-induced).	No. of injections.	Total of fresh tumour tissue injected (grams).	No. of tumours appearing monthly. 11	12	13	14	15	16	17	18	19	20	21	22	Total no. of tumours.	Average tumour age (months)	No. of mice alive at the earliest tumour appearance.	Average age of mice dying without tumours (months).
6	1F No. 36.23 (M)	5	0·25	0/1	..	..	..	0/2	..	..	..	..	..	0/2	..	..	..	5	16·5
6	„ „ 35.94 (M)	7	0·35	..	..	..	..	..	..	0/1	..	0/1	0/1	0/1	0/1	..	..	5	19·5
6	„ „ 37·39 (M)	7	0·35	0/1	..	..	..	..	..	..	0/1	0/1	..	..	0/3	..	..	6	19·0
12	„ „ 34·63 (M)	12	0·6	..	0/1	..	..	..	..	0/1	0/2	0/3	0/1	0/1	..	..	..	11	19·3
6	C3H „ 30 (Oe–M)	8	0·4	1/0	..	..	..	2/0	1/1	..	..	..	0/1	..	..	4	14·3	6	18·0

Numerators—number of mice developing tumours.
Denominators—number of mice dying without tumours.

TABLE II.—*Administration of Desiccated Induced Breast Tumour Tissues to C57 × RIII Susceptible Hybrid Female Mice.*

Experiment 2.

No. of mice.	Type of dried tumour tissue M (methylcholanthrene-induced) Oe–M (oestrone and methylcholanthrene-induced).	No. of injections.	Total of fresh tumour tissue injected (grams).	No. of tumours appearing monthly. 10	11	12	13	14	15	16	17	18	19	21	22	Total no. of tumours.	Average tumour age (months)	No. of mice alive at the earliest tumour appearance.	Average age of mice dying without tumours (months).
6	C3H No. 25 (Oe–M)	6	0·6	..	1/0	1/0	0/1	..	..	1/0	..	..	0/1	..	..	3	13	5	16
6	„ „ 27 (Oe–M)	6	0·6	..	..	1/0	..	1/0	0/1	..	0/2	0/1	..	..	..	2	13	6	16.8
6	„ „ 41 (Oe)	6	0·6	0/1	..	1/0	0/1	..	1/0	..	0/1	..	..	..	0/1	2	13·5	6	15·5
6	„ „ 38 (Oe)	4	0·4	1/0	..	..	..	..	2/0	..	1/0	0/1	..	0/1	..	4	14·3	6	19·5
6	C57 „ 23 (Oe–M)	3	0·3	..	..	..	..	..	..	0/1	0/2	..	..	0/1	..	..	..	4	17.8
6	„ „ 4 (M)	6	0·6	..	0/1	..	..	0/1	..	..	..	0/2	0/1	..	0/1	..	..	6	17

Numerators—number of mice developing tumours.
Denominators—number of mice dying without tumours.

and methylcholanthrene, one induced in a C57 strain breeding female with methylcholanthrene and one in a C57 strain male with oestrone and methylcholanthrene (Dmochowski and Orr, 1949) were tested for the presence of the mammary tumour agent.

Altogether 36 C57 × RIII hybrid females were employed in the experiment, and 6 mice were used for each of the tested tumours.

Mice of the first group were given C3H male No. 25 breast tumour tissue and mice of the second group C3H male No. 27 breast tumour tissue, both induced by administration of oestrone and methylcholanthrene. Each of the two groups received 6 injections, each of 1 c.c. of a distilled water suspension of dried tumour tissue, giving a total amount equal to 0·6 g. of fresh tissue.

Mice of the third group received 6 injections, each of 1 c.c. of a distilled water suspension of C3H No. 41 male tumour tissue induced with oestrone, corresponding to a total of 0·6 g. of fresh tissue.

Mice of the fourth group received 4 injections of C3H No. 38 tumour tissue induced in a male mouse with oestrone, giving an amount of dried tissue equal to 0·4 g. of fresh tumour tissue.

Breast tumour tissue induced in C57 male No. 23 by administration of oestrone and methylcholanthrene was given to mice of the fifth group in 3 injections, comprising a total of 0·3 g. of fresh tissue.

Mice of the sixth group were given 6 injections, each of 1·0 c.c. of a distilled water suspension of breast tumour induced in a C57 No. 4 breeding female with methylcholanthrene, an amount equal to 0·6 g. of fresh tissue.

The amount of tumour tissue administered in a given case necessarily varied with the size of the original tumour.

As can be seen from Table II, none of the C57 × RIII hybrid females, given repeated injections of breast tumour tissue induced in C57 low-breast-cancer strain mice either with oestrone and methylcholanthrene or with methylcholanthrene alone, developed breast cancer. The C57 × RIII hybrid female mice injected with C3H breast tumour tissue induced by oestrone and methylcholanthrene developed tumours ; breast tumours were also induced in C57 × RIII hybrid females with C3H breast tumour tissue obtained after administration of oestrone alone. The results of the present experiment taken in conjunction with the results obtained from Experiment 1 show that the mammary tumour agent is present in breast tumours induced in C3H high-breast-cancer strain male mice either by oestrogenic hormones alone, or by a combined treatment with methylcholanthrene and oestrogens. Mammary tumours induced in IF and C57 low-breast-cancer strain females after administration of methylcholanthrene as well as tumours obtained in C57 strain males after application of oestrone and methylcholanthrene do not seem to possess the agent.

DISCUSSION.

Biological tests for the presence of the mammary tumour agent in breast cancer induced in high- and low-breast-cancer strain mice by administration of methylcholanthrene alone, oestrone alone, or combined treatment have revealed a difference between induced breast tumours according to the strain in which they originated.

The mammary tumour agent is present in breast tumours induced in C3H high-breast-cancer strain males following treatment with oestrogenic hormones

and also after combined administration of these hormones and methylcholanthrene. This appears to be the first recorded demonstration of the mammary tumour agent in breast tumours of male mice from a high-breast-cancer strain. It was to be expected that male mice of C3H strain, whose females are very susceptible to the development of breast cancer, possess the agent, and indeed recent experiments (Andervont and Dunn, 1948) have shown the presence of the agent in the seminal vesicles of C3H males.

The results of the tests for the presence of the agent in breast tumours of C57 black low-breast-cancer strain male and female mice taken together with those of the tests of mammary tumours of IF low-breast-cancer strain females obtained after methylcholanthrene treatment, give a fair indication that breast cancer induced with methylcholanthrene in low-breast-cancer strain mice, both males and females, does not possess the mammary tumour agent. The method of repeated injections (Dmochowski, 1945) is very effective, and should have revealed the presence even of small amounts of the agent. Methylcholanthrene, therefore, does not induce the appearance of the milk factor in mice which do not possess it before treatment.

It is known from the experiments carried out by various workers that certain strains of mice show a greater susceptibility to the induction of breast cancer with methylcholanthrene than other strains. Kirschbaum and Bittner (1945), found that dba high-breast-cancer strain is very susceptible, while the C3H high-breast-cancer strain is less susceptible to the development of breast cancer following treatment with methylcholanthrene. Similar differences in the susceptibility to carcinogen-induced breast cancer may also be found in low-breast-cancer strains. Strain NH (Kirschbaum, Williams and Bittner, 1946) and also strain NHO (Strong, 1945) are susceptible, C57 black strain used by Kirschbaum, Williams and Bittner (1946) is not susceptible under the conditions of administration of methylcholanthrene employed by these workers. The sub-line of C57 black low-breast-cancer strain maintained in this department is susceptible, but to a less degree than IF low-breast-cancer strain mice, to the induction of breast cancer following the application of methylcholanthrene by the present method. Our C57 black strain mice do not develop breast cancer when given material containing the mammary tumour agent at the age of 4–6 weeks, even after large quantities of the material and in spite of increased hormonal stimulation (repeated pregnancies). This indicates that mature mice of this strain, though resistant to the mammary tumour agent, are susceptible to a different agent, namely, methylcholanthrene.

The development of breast tumours in both high- and low-breast-cancer strain mice after treatment with methylcholanthrene, the differences in the susceptibility to the development of these tumours between various strains which possess the agent as well as between strains which lack the agent, the absence of the agent in induced breast tumours of low-breast-cancer strain mice, although such tumours are similar in microscopical appearance to induced breast tumours of high-breast-cancer strain mice, support the conclusion that the action of methylcholanthrene in the induction of breast cancer is independent of the milk factor.

The results of the tests for the presence of the mammary tumour agent in breast tumours induced in low-cancer strain mice seem to support the conclusion that the type of susceptibility operative in the development of methylcholan-

threne-induced breast tumours differs from that involved in the origin of spontaneous breast cancer. The susceptibility to spontaneous breast cancer may be different from the susceptibility to chemically-induced breast cancer because mice which show low susceptibility to the mammary tumour agent may show a high susceptibility to methylcholanthrene, and vice versa.

The results of our tests for the presence of the milk factor in methylcholanthrene-induced breast tumours in low-breast-cancer strain mice show that methylcholanthrene does not induce the appearance of the factor in mice of strains which are known to lack it.

SUMMARY.

The present experiments have shown the mammary tumour agent to be present in breast tumours induced in C3H high-breast-cancer strain males, following application of methylcholanthrene and oestrogen, or oestrogen alone. Biological tests have not revealed the agent in breast cancer induced in C57 black low-breast-cancer strain males, after the application of oestrogen and methylcholanthrene. Breast tumours induced in C57 black and IF low-breast-cancer strain females by methylcholanthrene do not contain the mammary tumour agent.

The part played by the carcinogenic hydrocarbons in the induction of breast cancer in mice is discussed.

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SOME DATA ON THE DISTRIBUTION OF THE MILK FACTOR.

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AFTER the discovery of the mammary tumour agent or the milk factor in the milk of high-breast-cancer strain female mice (Bittner, 1936), numerous investigations were undertaken in order to ascertain its presence in various tissues and organs of normal mice of high-breast-cancer strains, and also in spontaneous breast tumours of these mice. For this purpose either implantation of various organs into suitable susceptible test mice was used, or extracts of these organs and tissues were made and injected into the test mice. In this way, on the basis of the development of breast cancer in the test mice, the milk factor was found to be present in the spleen (Bittner, 1939*a*; Andervont,

Shimkin and Bryan, 1942 ; Dmochowski, 1944*a*), thymus (Bittner, 1939*a*, *b*), lactating mammary tissue (Bittner, 1939*a* ; Andervont, Shimkin and Bryan, 1942 ; Andervont and Bryan, 1944), spontaneous breast tumour tissue (Bittner, 1941*a* ; Bryan, Kahler, Shimkin and Andervont, 1942 ; Visscher, Green and Bittner, 1942 ; Andervont and Bryan, 1944 ; Dmochowski, 1944*b* ; Barnum, Ball and Bittner, 1947), transplanted breast tumour tissue (Barnum, Ball and Bittner, 1947 ; Dmochowski, 1949), Harderian gland (Bittner and Watson, 1946). The factor is also present in whole blood (Woolley, Law and Little, 1941), and is more concentrated in cellular elements than in the serum (Bittner, 1945*a* ; Hummel and Little, 1949). The presence of the factor in some organs such as liver was doubtful (Bittner, 1941*b*, 1947), or was not ascertained as in the stomach milk, and there existed contradictory evidence about the transmission of the milk factor in the uterus of high-breast-cancer strain females (Fekete and Little, 1942 ; Andervont, 1945). All organs and tissues tested were those of high-cancer strain females, and it was only after the present experiments had been started that Andervont and Dunn (1948) reported the presence of the milk factor in the seminal vesicles of high-breast-cancer strain males, and quite recently Hummel and Little (1949) found the factor in the blood of high-cancer strain males.

In order to control some of the results of electron microscope studies of normal tissues of high- and low-breast-cancer strain mice, first reported by Passey, Dmochowski, Astbury and Reed in 1947, experiments were commenced during the second half of 1947, in order to ascertain the presence or the absence of the milk factor in : (1) normal organs of C3H high-breast-cancer strain males, (2) liver of C3H high-breast-cancer strain female mice, (3) normal organs of IF low-breast-cancer strain mice, (4) placenta and embryos of C3H high-cancer strain mice. In May, 1948, as the result of the electron microscope findings of large numbers of the characteristic particles in stomach milk of C3H mice (Passey, Dmochowski, Astbury and Reed, 1947–1948*a* ; Passey, Dmochowski, Astbury, Reed and Johnson, 1948), an additional experiment was carried out to test the stomach milk of 5-day-old C3H high-cancer strain mice to ascertain if the particles could be associated with the presence of the factor.

Experiment No. 1.

This experiment was carried out in order to give additional information about the results of electron microscope examinations of extracts of normal organs of high-breast-cancer strain mice in which, as in breast tumours of these mice, spherical particles presumed to be the milk factor itself had been described (Passey, Dmochowski, Astbury and Reed, 1947 ; 1947–1948*b*). Preliminary results of this experiment have already been reported (Dmochowski, 1947–1948, 1948*a*, 1948–1949*a*), and a full account is now presented.

Methods.

As the source of material the following organs of C3H high-breast-cancer strain males, 2–4 weeks old, were used : lungs, kidneys, spleen, thymus and heart. The organs from 14 C3H strain males were minced together, desiccated in the usual way (Dmochowski, 1946), and after a storage in the ice-chest for a fortnight used in the experiment. The desiccated organs were resuspended in distilled water in a proportion of 1:10, and injected subcutaneously every second

day in 0·5 c.c. quantities into the test mice. Each injection contained an amount of dried tissue equal to 0·05 g. of fresh tissue. Each mouse received 22 injections, corresponding to a total of 1·1 g. of fresh tissue. Eighteen C57 × R III susceptible hybrid females, 4–6 weeks old, were used. All females in this, as well as in the other experiments described, were forcibly bred by removing the first three litters within 24 hours of birth, and then allowed to breed in a normal way and kept under similar conditions on a diet of " rat-cake " cubes and oats, with an unlimited supply of tap water.

Results.

The results of the experiment are summarized in Table I. As can be seen from Table I, repeated administration of dried normal organs of 2- to 4-weeks-old C3H high-breast-cancer strain males induced breast tumours in 6 out of 15 susceptible test mice alive at the appearance of the first tumour.

Discussion.

It is not known how far the use of several organs of C3H high-cancer strain males, mixed and dried together, has influenced the results. There is no doubt, however, that C3H high-breast-cancer strain males possess the milk factor. Similar results have recently been reported by Andervont and Dunn (1948), who have demonstrated the presence of the factor in the seminal vesicles of C3H high-cancer strain males, maintained in their laboratories, and by Hummel and Little (1949), who have found the factor in the whole blood of dba high-breast-cancer strain males. It can be concluded that the milk factor is present not only in females but also in males of high-breast-cancer strains.

Experiment No. 2.

Methods.

Livers of 18 C3H high-breast-cancer strain females, 2–4 weeks old, were used as the source of material. The liver tissue was minced together, desiccated, and after one week's storage in the ice-chest, ground with distilled water in a proportion of 1:10, and injected subcutaneously every second day in 0·5 c.c. quantities into 15 4- to 6-weeks-old C57 × R III hybrid females. Each of the mice received 22 injections, giving an amount of dried material equal to 1·1 g. of fresh liver tissue.

Results.

The results are shown in Table II. Repeated injections of dried liver tissue of C3H high-breast-cancer strain females induced breast cancer in 2 out of 18 test mice alive at the appearance of the earliest tumour.

Discussion.

In previous experiments the method of repeated injections (Dmochowski, 1945) was found to be very effective. Therefore, in view of the large amount of material injected, it can be stated that the milk factor is present in the liver of young female mice of C3H high-cancer strain, but it is a matter of speculation whether it is present in small quantities or in an attenuated form. Hummel and

TABLE I.—*Administration of Dried Normal Organs of 2–4-weeks-old C3H High-breast-cancer Strain Male Mice to C57 × R III Hybrid Females.*

Number of mice.	Number of injections.	Total of fresh tissue (grammes).	Number of tumours appearing monthly. 10	11	12	14	17	18	19	20	21	Total number of tumours.	Number of mice alive at the earliest tumour appearance.	Average tumour age (months).	Average age of mice dying without tumours (months).
18	22	1·1	1/1	1/1	1/0	0/1	0/1	1/1	0/2	1/2	1/0	6	15	15·3	16·4

Nominators = Number of mice developing tumours.
Denominators = Number of mice dying without tumours.

TABLE II.—*Administration of Dried Liver Tissue from 2–4-weeks-old C3H High-breast-cancer Strain Female Mice to C57 × R III Hybrid Females.*

Number of mice.	Number of injections.	Total of fresh tissue (grammes).	Number of tumours appearing monthly. 9	10	11	15	16	17	18	19	21	24	Total number of tumours.	Number of mice alive at the earliest tumour appearance.	Average tumour age (months).	Average age of mice dying without tumours (months).
20	22	1·1	1/0	0/1	1/0	0/5	0/1	0/2	0/2	0/1	0/2	0/2	2	18	10·0	17·5

Nominators = Number of mice developing tumours.
Denominators = Number of mice dying without tumours.

TABLE III.—*Administration of Dried Normal Organs of 2–4-weeks-old IF Low-breast-cancer Strain Female Mice to C57 × R III Hybrid Females.*

Number of mice.	Number of injections.	Total of fresh tissue (grammes).	Number of tumours appearing monthly. 13	14	15	16	18	20	21	22	Total number of tumours.	Number of mice alive at the earliest tumour appearance.	Average tumour age (months).	Average age of mice dying without tumours (months).
12	12	1·15	0/1	0/2	0/1	0/3	0/1	0/2	0/1	0/1	0	—	—	17·1

Nominators = Number of mice developing tumours.
Denominators = Number of mice dying without tumours.

Little (1949) in their recently published experiments described a similar incidence of breast cancer in test mice following subcutaneous implantation of liver tissue or subcutaneous injections of minced liver tissue suspended in distilled water. They have put forward a suggestion that liver may carry the milk factor only by virtue of the blood it contains. The presence of small amounts of the factor in the liver tissue, at first not revealed in Bittner's experiments (1941*b*), will have to be further investigated in order to elucidate what part, if any, is played by the liver in the removal and destruction of the mammary tumour agent.

Experiment No. 3.

In connection with the presence of the milk factor in various tissues and organs of high-breast-cancer strain female mice, and the investigations of the presence of the agent in breast tumours induced in IF low-breast-cancer strain females after treatment with methylcholanthrene (Dmochowski and Orr, 1949), experiments were carried out to test normal organs of IF low-cancer strain females for the presence of the milk factor.

Methods.

The following organs of IF low-cancer strain females, 2–4 weeks old, were used as the source of material : kidneys, spleen, heart, lungs and thymus. These organs were minced together, desiccated, and stored for a fortnight in the ice-chest. Twelve C57 × RIII hybrid females, 4 weeks old, each were given 12 subcutaneous injections of a distilled water suspension of the dried organs. Altogether a total amount of material equal to 1·15 g. of fresh tissue was injected into each mouse.

Results.

Table III gives a summary of the results. No tumours developed in any of the test mice, although all of them survived beyond the age of appearance of the earliest tumours in the two previous experiments.

Discussion.

The results, taken together with the results of the tests for the presence of the milk factor in breast tumours induced in IF strain females following the application of methylcholanthrene (Dmochowski and Orr, 1949), give a fair indication that IF strain females do not harbour the milk factor. During the same interval of time breast tumours were obtained in test mice injected with similar quantities of dried normal organs and liver of C3H strain mice. This again points towards a conclusion that the factor is not present in the organs of IF low-breast-cancer strain females, at least in quantities which could be detected under the conditions of the test employed. Hummel and Little (1949) have reported similar results with the blood, liver and spleen of their D low-breast-cancer strain female mice.

Experiment No. 4.

Fekete and Little (1942) reported intra-uterine transmission of the milk factor by reciprocal transference of fertilized ova between female mice of high- and low-breast-cancer strains. Mice born from the transferred ova were nursed by mothers which gave birth to them. The original C57 low-cancer strain mice born from transferred ova developed an incidence of 50 per cent breast tumours

and three generations of their descendants gave an incidence of 73 per cent. The original dba high-cancer strain mice born from transferred ova had no tumours, while their progeny showed an incidence of 12 per cent breast cancer. Fekete and Little (1942) suggested that the intra-uterine transmission may have been responsible for their results. However, Bittner (1945*b*) reported an incidence of 1·4 per cent in 20 generations of foster nursed Strong A high-breast-cancer strain mice, and an incidence of 1·9 per cent in 10 generations of foster nursed C3H high-breast-cancer strain females. Andervont (1945) observed no breast tumours in 5 generations of mice descended from C3H young mice removed from the uterus of C3H high-cancer strain mothers and foster nursed by C57 low-cancer strain females. Should an intra-uterine transmission of the milk factor take place, as suggested by Fekete and Little (1942), the incidence of breast tumours in Bittner's experiments would have been much higher and Andervont would have observed breast tumours in mice of his experiments. The high incidence of breast tumours in the C57 low-cancer strain mice of Fekete and Little may be partially explained by the suckling of high-cancer strain mothers and also by the action of hormonal factors. The development of breast tumours in the dba strain mice is, however, more difficult to explain and requires further investigation. In connection with these contradictory observations an experiment was carried out in an attempt to elucidate this problem from a different angle.

Methods.

C3H high-breast-cancer strain embryos and placentas served as the source of material. They were removed from the uterus of several C3H high-cancer strain females in the last few days of pregnancy, and care was taken to wash off with distilled water all traces of maternal blood from the embryos, carefully separated from their placentas. All the embryos were then minced together and desiccated. Similarly all the placentas were minced and dried together. C57 × RIII hybrid females, 4–6 weeks old, mostly litter mates, were divided into two groups, each comprising 18 animals. Each mouse of the first group received 14 subcutaneous injections of a 1 : 10 distilled water suspension of desiccated embryos, giving a total equal to 0·7 g. of fresh tissue. Each mouse of the second group received a similar number of subcutaneous injections of a 1 : 10 distilled water suspension of dried placenta, giving a total of dried tissue corresponding to 0·7 g. of fresh tissue.

Results.

The results of the experiment are shown in Table IV. No breast tumours developed in any of the mice injected with dried embryos' tissue, in spite of the large quantities of the tissue injected, and only 2 breast tumours developed in test mice injected with dried placenta.

Discussion.

Hummel and Little (1949), in their recently published experiments, observed no tumours in test mice injected with either placentas of C3H high-cancer strain females or with the blood of their foetuses. The difference between their results and the present observation of 2 breast tumours in test mice injected with dried placentas of C3H high-cancer strain mice may be due to the large quantities of

TABLE IV.—*Administration of Dried C3H High-breast-cancer Strain Embryo and Placenta to C57 × R III Hybrid Females.*

Number of mice.	Type of tissue injected.	Number of injections.	Total of fresh tissue (grammes).	Number of tumours appearing monthly. 14	15	17	18	19	20	23	Total number of tumours.	Number of mice alive at the earliest tumour appearance.	Average tumour age (months).	Average age of mice dying without tumours (months).
18	Embryo	14	0·7	0/2	0/1	0/1	0/3	0/2	0/2	0/2	0	13	—	19·8
18	Placenta	14	0·7	1/1	0/1	0/1	1/6	0/1	0/1	0/3	2	16	16	18·7

Nominators = Number of mice developing tumours.
Denominators = Number of mice dying without tumours.

TABLE V.—*Administration of Dried C3H High-breast-cancer Strain Stomach Milk to C57 × R III Hybrid Females.*

Number of mice.	Number of injections.	Total of fresh tissue (grammes).	Number of tumours appearing monthly. 6	7	9	10	11	14	15	16	17	Total number of tumours.	Number of mice alive at the earliest tumour appearance.	Average tumour age (months).	Average age of mice dying without tumours (months).	Number of mice alive after 18 months from the beginning of the experiment.
12	10	0·95	1/0	1/0	1/0	2/0	1/0	1/0	2/0	0/1	1/0	10	12	11·3	16	1
19	1	0·1	–	0/2	–	1/0	0/1	3/0	1/0	–	–	5	19	13·4	8·3	11

Nominators = Number of mice developing tumours.
Denominators = Number of mice dying without tumours.

tissue injected. The results permit the conclusion that the milk factor is not present in C3H high-cancer strain embryos before their birth and is present, perhaps only in very small quantities, in the placenta of C3H high-cancer strain females. The method of repeated injections (Dmochowski, 1945) should have revealed the presence of small amounts of the milk factor in the embryos, as it has revealed it in the liver tissue or in the placentas. It is not known whether this is due to some unknown factor or factors which neutralize the milk factor in the placenta. Hummel, Little and Eddy (1949) have published results which seem to indicate that the mature placenta is the site of the neutralization or inhibition of the milk factor. It seems that there is no intra-uterine transmission of the milk factor, and the present results corroborate the findings of Bittner (1945b), and of Andervont (1945), as well as the recent observations by Hummel and Little (1949).

Experiment No. 5.

Electron microscope examination of extracts of milk obtained from stomachs of 5-day-old C3H offspring had revealed the presence of the same spherical particles (Passey, Dmochowski, Astbury, Reed and Johnson, 1948) as those which had been observed in extracts of normal and malignant tissues of mice of several high-breast-cancer strains. Biological tests were, therefore, carried out to ascertain whether the stomach milk containing these particles possesses the breast tumour inducing property. Preliminary results of these tests, already briefly reported (Dmochowski, 1948*b*, 1948–1949*b*), are now described in detail.

Methods.

In a further search for a good supply of the milk factor, stomach milk of 5-day-old C3H high-breast-cancer strain mice was examined. The stomach milk was obtained from young C3H high-cancer strain mice by killing the animals under ether anaesthesia half-an-hour after they suckled their mothers, and was desiccated in the usual manner. The test mice, 4–6 weeks old, were divided into 2 groups. One group received 10 subcutaneous injections of a 1 : 10 distilled water suspension of desiccated stomach milk, equal to a total of 0·95 g. of fresh milk, the other group received 1 injection of a similar suspension of the stomach milk, corresponding to 0·1 g. of fresh milk.

Results.

Table V summarizes the results so far obtained. As can be seen, a higher number of breast tumours developed in the test mice given repeated doses of the stomach milk as compared with the number of tumours in animals injected with a single dose of the desiccated stomach milk. Also the breast tumours developed at a later age in the latter group of test mice than in the former group.

Discussion.

The present results show that the milk factor is present in the stomach milk of young 5-day-old C3H high-breast-cancer strain mice, which is therefore a good source of the mammary tumour agent. Similar results have been reported by Hummel and Little (1949),who found the stomach contents of 4- to 11-day-old Strong A and C3H strain mice a source of an active mammary tumour agent.

SUMMARY.

1. Normal organs, such as liver, lungs, spleen, kidneys, and heart of 2- to 4-weeks-old C3H high-breast-cancer strain males contain the milk factor as shown by the development of breast tumours in susceptible C57 × R III hybrid females, injected with extracts of the desiccated organs of these animals.

2. The mammary tumour agent is present only in small amounts in the liver of 2- to 4-weeks-old C3H high-cancer strain females.

3. Normal organs of young IF low-breast-cancer strain females do not harbour the milk factor. Breast tumours were not observed in test mice given repeated injections of extracts of these organs.

4. While embryos of C3H high-cancer strain female mice do not show the presence of the factor, placenta of these mice contains the milk factor only in very small amounts.

5. Stomach milk of 5-day-old C3H high-cancer strain mice has been found to be a good source of the mammary tumour agent.

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THE DEVELOPMENT OF MALIGNANCY IN EXPERIMENTALLY-INDUCED ADENOMATA OF THE THYROID.

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A PREVIOUS communication (Hall, 1948) reported the induction of multiple adenomata in the thyroid of rats by the administration of 10–15 mg. of 2-acetylaminofluorene (A.A.F.), followed by methyl-thiouracil treatment for periods up to 26 weeks. Methyl-thiouracil (0·01 per cent in drinking water) given for similar periods induced occasionally single adenomata. In this paper an account will be given of the changes occurring in the thyroids of rats similarly treated, the administration of methyl-thiouracil being continued for 52–94 weeks.

Several authors have described in detail the pathogenesis and histology of the benign experimental tumour of the rat thyroid (Bielschowsky, 1945; Griesbach, Kennedy and Purves, 1945; Money and Rawson, 1947; Laqueur, 1949). The effects of prolonged treatment with goitrogenic agents leading to the formation of malignant tumours of the thyroid have been described by Purves and Griesbach (1946, 1947). Cancers of the thyroid have been obtained also by the simultaneous administration of A.A.F. and an anti-thyroid compound (Bielschowsky, 1944; Paschkis, Cantarow and Stasney, 1948). However, little is known about the changes preceding the transformation of thyroid adenomata into cancers, or of the possible role played by A.A.F. in the change to malignancy.

MATERIAL AND METHODS.

Albino rats of both sexes, 6–8 weeks old, were used. They came from the same colony as the animals used in the experiments previously reported (Hall, 1948). The rats were fed the same diet, and the concentration of the methyl-thiouracil in their drinking water was again 0·01 per cent. Six doses of 2·5 mg. of A.A.F. were given by stomach tube to 28 rats (Group A) during the first week of the experiment. Subsequently these animals received methyl-thiouracil, 22 up to the day they were sacrified, 6 rats being killed 3–4 weeks after the withdrawal of the goitrogen.

Sixteen rats (Group B) received methyl-thiouracil only, the A.A.F. treatment being omitted. Rats from both groups were killed at intervals between the 53rd and 95th week. A thorough post-mortem examination was made in order to detect the presence of neoplastic lesions in organs other than the thyroid gland. The material chosen for histological study was fixed in formol saline or in Zenker solution, cut at 7μ and stained with haematoxylin-eosin or Weigert-Van Gieson. Numerous sections representing different parts of the thyroid gland were studied.

Four large blocks of lung tissue, chosen at random from each rat killed after 76 weeks of methyl-thiouracil treatment, were cut in serial sections and searched for the presence of metastases.

RESULTS.

52–74 *weeks.*

The thyroids of the rats killed after 12–16 months of continued stimulation were greatly enlarged and of deep red colour. Generally naked-eye inspection failed to detect obvious differences between the thyroids of animals belonging to Group A or B. Microscopically the glands of the rats which had received A.A.F. showed features which were not present or less pronounced in the controls.

Many adenomata of varying size and structure were seen in all thyroids of Group A. They were so numerous that nearly every section studied revealed the presence of several independent neoplastic nodules. On the average they were considerably larger than similar nodules found in the controls. Here several sections had to be examined to obtain evidence of their presence. At this stage the adenomata were devoid of features characteristic of malignancy. In Group A large cysts filled with colloid made their appearance, having diameters of several millimetres and compressing frequently the surrounding hyperplastic tissue.

Most prominent were enormous blood sinuses filled with erythrocytes especially near or within the adenomata. The engorged vessels separated groups of follicles from the contact with acini formerly adjoining them.

Most striking were changes seen in the hyperplastic thyroid tissue, occurring to a varying degree in rats of both groups killed during the 53rd–63rd week, animals of Group A being generally more affected than the controls. Widespread desquamation of degenerating thyroid epithelium was the characteristic feature of this process. The cytoplasm of the thyroid epithelium became highly vacuolated, so that the epithelial cells were hardly stained by eosin. The majority of nuclei were pycnotic, and many epithelial cells had lost their connection with the basal membrane. In consequence many follicles collapsed and only the capillaries surrounding them remained. Fig. 1 illustrates an advanced stage of this condition. Here the degenerative changes had progressed so far that in parts of the gland the alveolar structure had completely disappeared. Like in advanced post-mortem changes the epithelial cells, having lost all cohesion, were lying scattered in a network formed by the better preserved capillaries. These desquamated cells became round, had pycnotic nuclei to which rests of cytoplasm adhered. In comparison the adenomata, although showing some degenerative changes, were better preserved (Fig. 1). When the nodules were more heavily affected the periphery suffered most, so that the boundaries between adenoma and thyroid tissue became ill defined.

Many thyroids of rats killed between the 70th and 74th week showed differences in the size of the two lobes of the gland, one being more enlarged than the other. In some instances bluish-red areas, corresponding to large cysts, were visible on naked-eye inspection. Histological examination revealed a recovery of the thyroid from the degenerative changes described above. The adenomata were now very numerous in the glands of both groups, being perhaps slightly larger in Group A. The adenomata compressing the hyperplastic thyroid tissue accounted for most of the substance of the gland. Thickening of the capsule, from which bundles of connective tissue radiated into the parenchyma, was a

frequent finding. The sequels of haemorrhages, as replacement of glandular by connective tissue and presence of numerous macrophages filled with pigment, were seen in many glands. The adenomata showed now features indicating a possible change in their benign character. Signs of beginning of anaplasia, increasing basophilia of the cytoplasm of tumour cells and small foci of cells arranged in a disorderly fashion were occasionally seen, but definite proof of malignancy could not be obtained at this stage. Mitoses were extremely rare.

76–94 *weeks.*

Most of the thyroids of both groups of rats killed after 76 or more weeks of continuous administration of methyl-thiouracil contained macroscopically recognizable nodules and cysts. In contrast to the uniformly red glands of animals killed at an earlier date now patches of yellowish-white tint appeared associated with nodules of macroscopic size. These alternated with areas of bluish colour due to the presence of cysts approaching the capsule.

In one animal killed during the 76th week of the experiment a metastasis was found in the lung (Fig. 2). The thyroid of this rat showed most of the lesions which are characteristic of the histological picture at this stage. The gland contained multiple adenomata of varying size and structure. Some were of the papillary type, others were formed by follicles, the larger of which contained abundant colloid. In the centre of the right lobe the thyroid tissue was replaced by dense connective tissue, through which cords of highly anaplastic tumour cells were crossing. These small cells had dark round or oval nuclei rich in chromatin, their cytoplasm was scanty and cell boundaries were not always clearly distinguishable. They rarely formed microfollicular structures. These changes were found in most rats killed after similar periods of methyl thiouracil treatment. Fig. 3 illustrates the anaplastic changes, and Fig. 4 depicts another new feature seen at this stage—projections of neoplastic thyroid follicles into large sinuses. Only a single layer of endothelial cells separated these neoplastic structures from the lumen of the engorged blood vessels.

Fifteen rats, eight of Group A and seven of Group B, were sacrified between the 77th and 95th week. In seven instances evidence of malignancy was obtained. Metastases were found four times (Fig. 2, 4), penetration of the capsule twice (Fig. 5), and invasion of the parathyroid once (Fig. 6). Only one animal of

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EXPLANATION OF PLATES.

FIG. 1.—Thyroid after 58 weeks of stimulation (Group A). Advanced degenerative changes of the glandular tissue. The adenoma is less affected. × 90.

FIG. 2.—Metastasis in lung (Rat 12, Group A). × 45.

FIG. 3.—Anaplastic changes in thyroid tumour (Rat 15, Group A). × 300.

FIG. 4.—Projections of neoplastic thyroid follicles into large blood sinus (Rat 12, Group A). × 690.

FIG. 5.—Penetration of capsule by malignant adenoma (Rat 10, Group B). × 45.

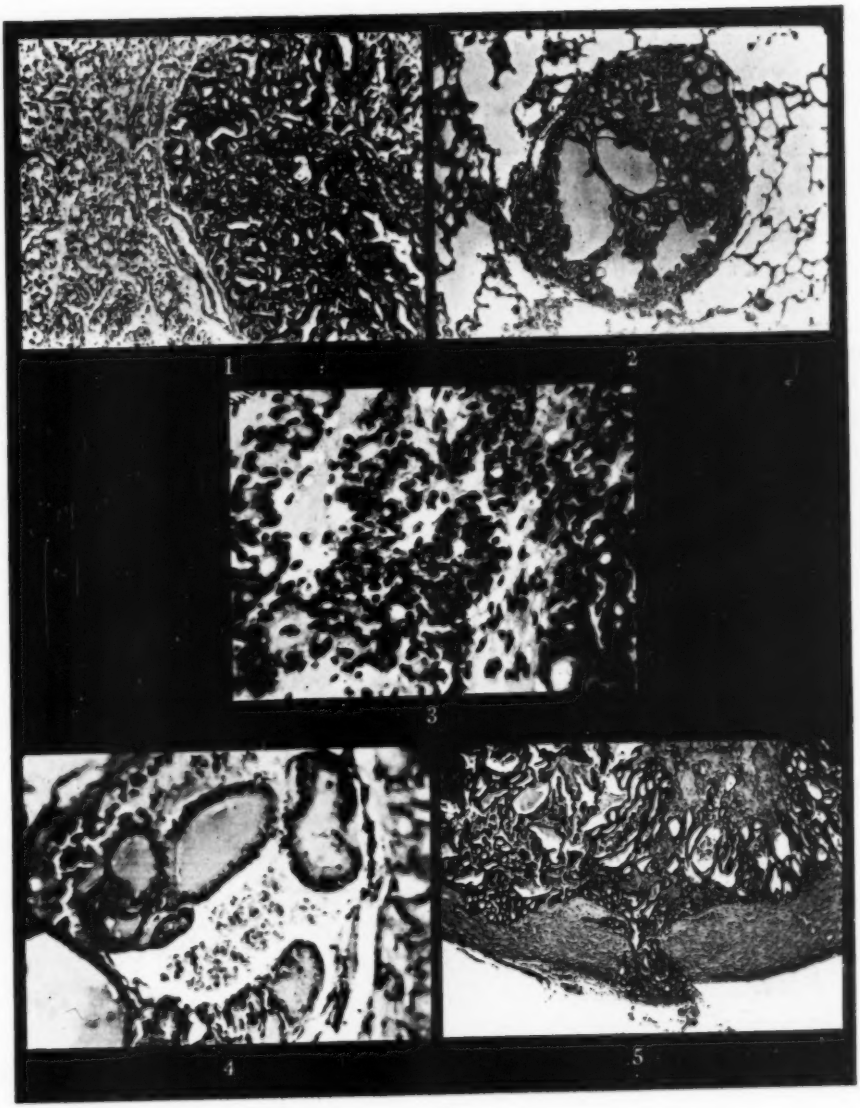
FIG. 6.—Invasion of parathyroid by tumour with papillomatous structure (Rat 20, Group A). × 45.

FIG. 7.—Small rim of hyperplastic thyroid tissue surrounded by neoplastic tissue. Compare with Fig. 9 (Rat 21, Group A). × 90.

FIG. 8.—Squamous keratinizing epithelium lining cystic space (from a thyroid tumour of Rat 18, Group A). × 145.

FIG. 9.—Subpleural metastasis of macroscopic size (Rat 21, Group A). × 45.

FIG. 10.—Thyroid 26 days after withdrawal of methyl-thiouracil. Signs of involution in adenoma and in thyroid tissue. × 90.



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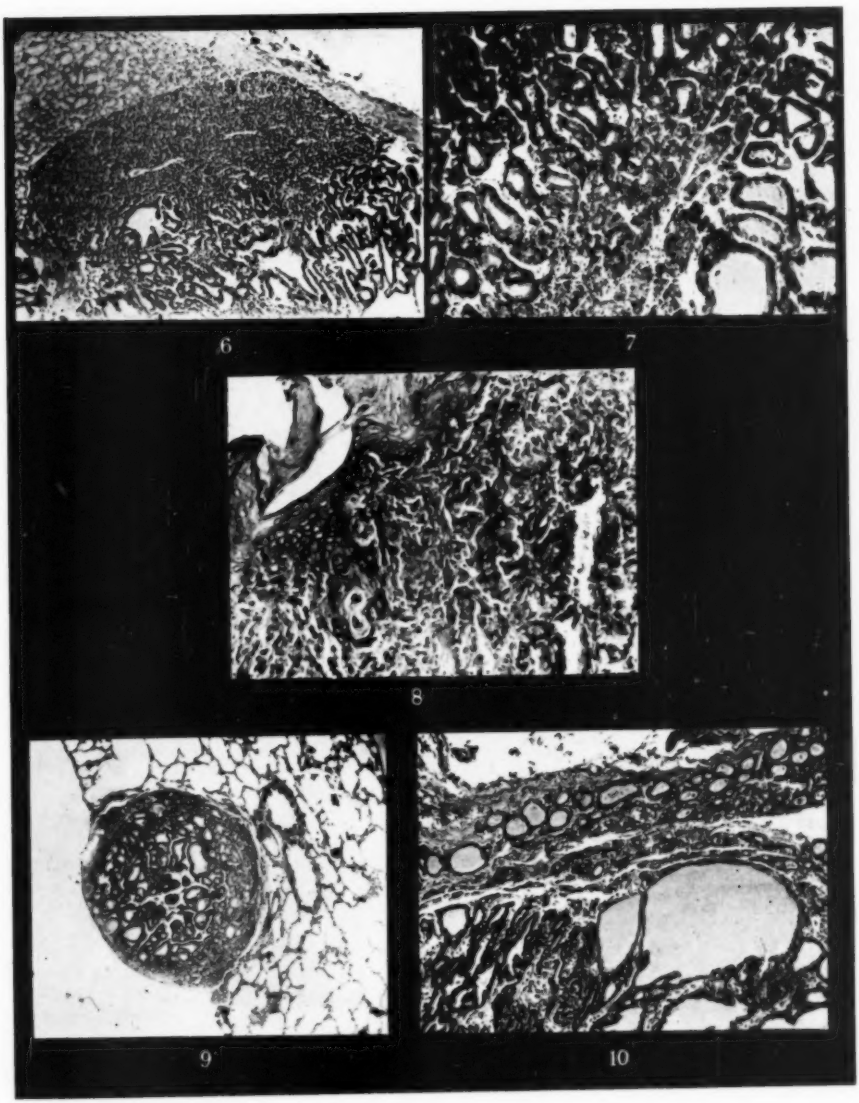

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Group A had macroscopically visible multiple secondaries situated on the surface of the lung. The other malignant lesions were discovered by histological examination. The results are summarized in Tables I and II.

TABLE I.—*Occurrence of Histological Signs of Malignancy in Group A.*

Rat No.	Sex.	Duration of experiment (weeks).	Thyroid.
1–9	♀	53–74	No signs of malignancy.
10	,,	76	,, ,,
11	,,	76	,, ,,
12	,,	76	Metastasis in lung.
13	,,	76	No signs of malignancy.
14	,,	77	,, ,,
15	,,	78	Penetration of capsule.
16	,,	78	No signs of malignancy.
17	,,	78	,, ,,
18	,,	78	,, ,, *
19	,,	78	Metastasis in lung.
20	,,	78	Invasion of parathyroid.
21	,,	94	Metastasis in lung.
22	,,	94†	No signs of malignancy.

* Squamous metaplasia. † Adenoma of lung.

TABLE II.—*Occurrence of Histological Signs of Malignancy in Group B.*

Rat No.	Sex.	Duration of experiment (weeks).	Thyroid.
1–9	♂	52–70	No signs of malignancy.
10	♀	78	Penetration of capsule.
11	,,	78	No signs of malignancy.
12	,,	78	,, ,,
13	,,	78	Metastasis in lung.
14	,,	78	No signs of malignancy.
15	,,	78	,, ,,
16	♂	94	,, ,,

Macroscopically no differences were seen between thyroids, which on histological examination were found to have given origin to metastases, and between the glands of animals free from secondaries. Histological study of the tumours bearing signs of malignancy failed to reveal the presence of specific character associated with metastases or invasive growth. In their cytology and general structure the malignant neoplasms (Fig. 5–7) did not differ from many nodules with well-defined borders. As already mentioned, after the 70th week of the experiment anaplastic changes were of frequent occurrence. At this stage it became obvious that the larger nodules had been formed not only by expansive growth but also by confluence of formerly individual adenomata. Frequently only a small rim of compressed hyperplastic thyroid tissue was seen isolated in areas otherwise completely comprised of neoplastic growth (Fig. 7). With increasing size regressive changes in the central part of the adenomata became

more frequent, areas of dense connective tissue replacing epithelial structures. This connective tissue originated from the adventitia of larger vessels with thickened walls or from bundles of fibres derived from the capsule. In one rat of Group A killed during the 78th week a large cyst measuring 3 × 2 mm. was found. In contrast to the colloid cysts seen frequently in the thyroids of rats of Group A and B this cyst was lined by squamous keratinizing epithelium and the material filling its cavity consisted of keratin and cell debris. Projections of squamous epithelium grew into the substance of the neoplastic tissue surrounding the cyst (Fig. 7). There were no signs of inflammation, past or present, nor of degenerative changes in the tumour containing this cyst. It seemed possible that the squamous metaplasia developed after formation of a cavity in consequence of a haemorrhage or a massive necrosis, but the possibility could not be excluded that accumulation of keratin in this rather anaplastic tumour gave rise to increased pressure with subsequent death of cells.

In most tumours very few mitoses were present; they were equally rare in neoplasms showing invasive growth as in those having well-defined boundaries. Even in areas such as depicted in Fig. 5 and 6 only an occasional cell division could be found. However, mitoses were numerous in the macroscopically visible metastases.

Six rats of Group A were killed during the 81st to the 82nd week of the experiment, three to four weeks after the withdrawal of methyl-thiouracil. The thyroids of these animals differed from those sacrificed after 78 weeks of uninterrupted administration of the goitrogen. The thyroids were no longer hyperaemic, engorged blood sinuses remaining only in some of the adenomata, and most of the hyperplastic thyroid tissue had returned to its normal state. The return of the glandular elements to the condition of normal activity did not proceed at a uniform rate. Apart from individual variations there existed also differences in the degree of involution inside the same gland. Whereas the follicles in the periphery of the thyroid were already lined by low cuboid epithelium and contained abundant colloid, towards the centre some follicles were still formed by tall cells with vesicular nuclei and contained scanty colloid. As before, neoplastic tissue comprised the greater part of the thyroid. The better differentiated as well as the undifferentiated tumours appeared healthy and no evidence for degeneration was found except in one instance. In this thyroid one of the several adenomata present had been completely destroyed by necrosis. In another rat killed also 26 days after the withdrawal of methyl-thiouracil, a tumour was found which showed an unusual degree of mitotic activity. Indeed, more cell divisions were seen in this tubular adenoma than in any other neoplasm examined during this investigation. At the same time many of the better differentiated, obviously benign adenomata underwent a similar change as the non-neoplastic tissue. The epithelium tended to become low and the accumulation of colloid increased (Fig. 10). Attempts have been made to transplant neoplastic thyroid tissue into other rats. Two of the animals killed three weeks after the withdrawal of the methyl-thiouracil were used as donors, others belonged to Group A or B. Here it will suffice to state that takes were obtained independently whether or not the donors were killed when still receiving methyl-thiouracil.

The livers of the rats treated with A.A.F. were found to be free of neoplastic lesions. The only tumour found in an organ other than the thyroid was a minute

adenoma of the lung occurring in a rat of Group A killed during the 95th week. This type of neoplasm, although readily induced by prolonged administration of A.A.F., has not been found so far in normal animals of our stock. The possibility however cannot be excluded that the lung tumour found when searching for metastases from cancers of the thyroid was a spontaneous neoplasm. In the same animal the uterus, especially the cervix, was grossly enlarged. Histological examination of this organ revealed only an extraordinary symmetrical hyperplasia of muscle and connective tissue without neoplastic changes.

DISCUSSION.

From previous experiments it is known that the presence of latent neoplastic cells in the thyroids of rats treated subsequently with a goitrogenic agent is responsible for the rapid development of adenomata in such glands. As shown in this paper, the effects of the administration of A.A.F., the inductor of these latent neoplastic cells, are still recognizable after 64 weeks of continued stimulation with methyl-thiouracil. Comparison between the thyroids of two groups of rats stimulated for similar periods reveals that the glands of the group treated with A.A.F. during the first week of the experiment contain more and larger adenomata. These differences diminish in proportion to the duration of uninterrupted stimulation to which the thyroids are subjected. At 18 months the thyroids of rats who had received A.A.F. are similar to those of animals treated with methyl-thiouracil only. At this time histological signs of malignancy are also present. Metastases, invasion of neighbouring structures and penetration of the capsule were used as criteria for establishing the diagnosis of malignancy. Seven instances of this condition were found, five in Group A and two in Group B. The greater frequency of cancers in the former group might easily be due to chance. Possibly some malignant changes have escaped detection because it was impossible to cut all lungs and all thyroids completely in series. By examining numerous sections of the greatly-enlarged glands as well as serial sections of four blocks of lungs of each animal killed during the 76th–94th week it was hoped to minimize this inadequacy.

The fact that cancers appeared in both groups at about the same time indicates that the change to malignancy occurred independently whether A.A.F. was given or not. Under the condition of the experiment the action of the carcinogen ceased, once it had played its role as an initiator. The fate of the neoplastic cells created by A.A.F. seems entirely dependent on the duration of the stimulation they were subjected to. Continued hormonal stimulation, although relatively slow in inducing benign neoplastic lesion in thyroids free of latent tumour cells, nevertheless leads to the appearance of cancers after the same interval in which malignancy was found in the glands of A.A.F.-treated rats.

Gorbman (1947) studying the effects of thiourea on the thyroid of mice has questioned the validity of some of the conclusions reached by Bielschowsky (1944) and Purves and Griesbach (1947), who worked with rats, a species which reacts quite differently to the action of goitrogens. These authors considered metastases, penetration of the capsule and invasive growth as proof of malignancy and therefore called tumours having thesecha racteristics carcinomata. Gorbman's assumption that in mice mechanical forces are mainly responsible

for penetration of the capsule and the presence of intravascular thyroidal follicles seems quite feasible, but does not satisfactorily explain the lesions depicted in this paper or in the communications of Purves and Griesbach. One of the great difficulties students of the action of goitrogens in mice have to deal with is the presence of pulmonary metastases without evidence of a primary tumour in the thyroid (Gorbman, 1947 ; Dalton, Morris and Dubnik, 1948). This anomaly does not exist in the rat. Here definite tumours are found, the structure of which can be compared with that of the surrounding tissue and with the metastases. In the rat normal thyroid tissue has never been found in the lung, and when thyroid tissue is discovered in this organ after prolonged treatment with a goitrogen it always shows signs of neoplasia. To us it seems highly improbable that penetration through an obviously thickened capsule, as depicted in Fig. 5, can result from pressure only. Even if the formation of tumour emboli can be explained by the particularly vulnerable barrier between neoplastic thyroid tissue and engorged blood sinuses, such embolic cells have to penetrate the walls of pulmonary arteries in order to establish a metastasis. These secondaries can reach diameters far in excess of that of the largest blood vessels of the rat, and the significance of their location in lung tissue adjoining a pulmonary artery has been stressed by Purves and Griesbach (1947).

In one minor point our results seem to differ from those of Purves and Griesbach (1947). These authors found a " definite epithelial change towards regression in three tumours of carcinomatous type " after 3 weeks' treatment with thyroid digest subsequent to 22½ months of thiourea administration. Treatment with thyroid hormone hastens the return of the thyroid to normal activity, and is a far more effective procedure to obtain involution of the gland than the withdrawal of the goitrogen. In our material signs of involution were found in the better differentiated adenomata ; some of the more undifferentiated neoplasms, however, showed signs of active growth. It seems, therefore, that the latter type of tumour cell can multiply although the stimulation has ceased. The terms involution or regression have been used by Purves and Griesbach and also in this paper to describe the return of the activated thyroid epithelium to its resting state. These terms do not imply degeneration or disappearance of experimental thyroid tumours.

The question arises whether the breakdown of the thyroid tissue preceding by three to four months the appearance of malignant changes plays any rôle in their pathogenesis. When the Leydig cells of certain strains of mice are stimulated by oestrogens for prolonged periods, they first become hyperplastic, then degenerate, and finally become neoplastic. The process is a discontinuous one (Hooker, 1948). In the case of the thyroid neoplastic nodules appear long before exhaustion occurs, and during the phase when degenerative changes in the thyroid are at their maximum, healthy neoplastic cells can always be found. It seems, therefore, that the experimental carcinomata of the thyroid are the result of a continuous process.

To conclude, the histological appearance of the experimental thyroid tumours described in this paper warrants the diagnosis of carcinoma in seven instances. Morphological signs of malignancy however do not prove that the state of autonomy has been attained by a tumour. The problem of autonomy of the experimental tumours of the rat thyroid has been attacked by studying their behaviour on transplantation and will be reported in a separate paper.

SUMMARY.

A comparative study has been made of the changes occurring in the thyroids of rats after 52–94 weeks of administration of methyl-thiouracil alone or subsequently to treatment with 15 mg. of A.A.F. It was found that the animals which had received A.A.F. had more numerous and generally larger adenomata when killed during the 52nd–64th week of the experiment. These differences disappeared gradually, and after 1½ years the glands of the A.A.F.-treated rats became indistinguishable from those of the controls. At this time morphological signs of malignancy appeared in both groups. These findings can be explained by the assumption that the small dose of A.A.F. given acts only as an initiator, accelerating the appearance of benign multiple adenomata, but does not hasten the development of malignancy. The formation of the carcinomata seems to be the result of continuous stimulation.

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STUDIES ON EXPERIMENTAL GOITRE: THE TRANSPLANTABILITY OF EXPERIMENTAL THYROID TUMOURS OF THE RAT.

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THE induction of tumours in the rat thyroid by means of goitrogenic compounds has been described in previous communications (Griesbach, Kennedy and Purves, 1945; Purves and Griesbach, 1946; Purves and Griesbach, 1947), and it was suggested that these neoplasms were the result of prolonged stimulation of the thyroid by excessive amounts of pituitary thyrotropic hormone. Money and Rawson (1947) confirmed these results, and reached similar conclusions about the mechanism responsible for tumour production. Administration of

the carcinogen, 2-acetamidofluorene, previously to or simultaneously with the goitrogen gave rise to an increased number of tumours and accelerated the rate of their formation (Bielschowsky, 1944, 1945; Paschkis, Cantarow and Stasney, 1948; Hall, 1948). In some of these tumours all the histological features of malignancy were found, irrespective of whether acetamidofluorene had been combined with the goitrogen or not, and the term cancer has been used in the description of such neoplasms. This paper reports the behaviour of experimental thyroid neoplasms on transplantation.

MATERIAL AND METHODS.

The "goitrogen induced" tumours were from old animals of both sexes which had been treated with methylthiouracil (0·01 per cent in the drinking water) for 18–24 months. The "carcinogen induced" tumours were from animals which had received 2·5 mg. of 2-acetamidofluorene daily for six days prior to the methylthiouracil administration of 18–20 months' duration.

Three types of recipient animals were used. These were: (*a*) normal rats 6–12 weeks old, of both sexes; (*b*) rats receiving methylthiouracil (0·01 per cent in the drinking water), the administration of the methylthiouracil being commenced in the week prior to inoculation; (*c*) partially or totally thyroidectomized rats which had been operated on in the week prior to inoculation. All animals were maintained on a diet consisting of bran 30 g., pollard 25 g., bone meal 15 g., pea meal 15 g. and maize meal 15 g.

For the primary transplantation areas of a paler appearance were taken from the donors' thyroids, as experience had shown that these were the most likely to contain neoplastic tissue. This material, in the form of small pieces, was implanted subcutaneously into the flank of the recipient rats. For secondary transplantation, tumours which had developed from primary transplants were either treated as for primary transplantation or, in some experiments, were finely cut with scissors and injected as a suspension.

RESULTS.

Donors.

Of the twelve donor animals used for this study, eight were treated with goitrogen alone and four had received AAF in addition. All animals had grossly enlarged tumorous thyroids. There was no histological difference between "goitrogen" and the "carcinogen induced" tumours. Two representative examples are shown in Fig. 1 and 2.

First generation of transplants.

Twelve tumours were successfully transplanted into rats receiving methylthiouracil. Sixty rats were inoculated, 38 of which (63 per cent) developed tumours at the site of implantation. Some of the transplants became palpable after three weeks, others were not detected until two months after inoculation. In some no transplants were palpable, but tumours were found at autopsy. This different rate of growth was found even in rats which had been inoculated with material from the same primary thyroid tumour. In some rats large tumours, up to 3·7 g. in weight, were obtained 4–6 months after inoculation. In others the graft did not reach such dimensions. Growth stopped after a few weeks, and

there was no apparent increase in size during a period of observation of up to nine months. The larger tumours became closely adherent to the surrounding tissue, and had an extraordinarily rich blood supply visible in some instances through the intact skin.

On histological examination of the tumours a considerable diversity of structures was seen, not only in grafts from different sources, but even within a single graft. Nodules composed of closely packed cells with pale nuclei, arranged in small acini or in solid masses, alternated with large follicles lined by cells with hyperchromatic nuclei. Cysts into which neoplastic epithelium grew in a papillomatous fashion were also present. All these structures were present in the original thyroid tumours (Fig. 1–5). Mitoses were most frequent in the nodular parts, but were also found in the follicular type of tumour. As well as tissue having the unmistakable characteristics of neoplasia (Fig. 6), some transplants contained areas of alveoli indistinguishable from the follicles of simple hyperplastic goitre. These were derived from normal thyroid tissue included in the grafts. In the slowly growing transplants, which reached large dimensions after six months, there was a tendency to form large cysts of irregular shape, filled with an eosinophilic colloid material (Fig. 7). These were lined with epithelium, which ranged from tall cylindrical to low cuboidal or flat in a single cyst. In areas showing a trabecular structure, the single layer of tall epithelium was frequently replaced by densely crowded cells suggesting stratification (Fig. 8). In two rats, invasion of the muscle was seen. Fig. 9 shows the thyroid tumour breaking up and destroying the striated muscle fibres. In normal animals none of the grafts derived from the same material grew, and at autopsy no trace was found of the grafted tissue.

Second generation of transplants.

Like the original thyroid tumours, the first generation of grafts could be successfully transplanted only into rats with a thyroxine deficiency, induced in this case surgically as well as by methylthiouracil treatment. Sixty-eight rats were inoculated with finely cut tumour tissue: 18 normal rats, 18 methylthiouracil treated and 32 thyroidectomized. Fourteen tumours developed in the methylthiouracil group (77 per cent), 16 in the thyroidectomized group (50 per cent) and none in the normals. The growth rate of the tumours in this generation also varied considerably, e.g. six grafts obtained from the same parent material ranged in weight from 100 to 3600 mg., after six months in methylthiouracil treated rats. In thyroidectomized animals grafts became palpable at about the same time as those in the methylthiouracil group, but in contrast did not reach weights over 1000 mg.

Histologically, some of the tumours of the second generation showed the same multiplicity of structures as the parent material. Others, however, showed a tendency to a more uniform follicular type of structure (Fig. 10). Differences were seen in the grafts removed from thyroidectomized and methylthiouracil treated rats. The acinar epithelium of the latter group was higher, with rounded nuclei and vacuolated cytoplasm. In grafts from the thyroidectomized group the cells were closely packed and compressed into wedge-like shapes, indicating a period of past activity. The nuclei had a strong affinity for haematoxylin (Fig. 11). The amount of eosinophilic material within the acini was much greater in the tumours from thyroidectomized animals. Grafts from methyl-

thiouracil-treated animals showed, as well as acini containing a pale vacuolated colloid, spaces lined with endothelial cells and filled with erythrocytes. These abundant and greatly dilated blood sinuses formed an integral part of the architecture of these tumours. The intimate relationship between these blood sinuses and the trabecular arrangement of the acinar epithelium is also shown in Fig. 8.

In one instance a tumour which, in the first generation, had grown more rapidly than the other transplants showed a further acceleration of growth rate in the second generation. Associated with this change in growth rate was a change in the histological appearance. The follicular structure was replaced in parts by a solid growth of cells with swollen nuclei and scanty cytoplasm and mitoses were exceedingly numerous (Fig. 13). Another tumour gave a second generation graft which grew slowly and had the histological appearance of a colloid goitre (Fig. 14). In contrast, the thyroid of this animal showed the picture to be expected after treatment with methylthiouracil.

In order to avoid repetition, it may be stated that the same percentage of takes as in the second generation of transplants occurred in the third and fourth generation. No changes in transplantability and growth-rate were observed.

Metastases.

In one animal post-mortem examination revealed the presence of a small nodule (2 mm. diameter) attached to the peritoneal surface of the diaphragm near its costal margin. It had the same histological appearance as the two

EXPLANATION OF PLATES.

FIG. 1.—Thyroid of rat treated with methylthiouracil for 24 months, showing two tumour nodules, one a microfollicular adenocarcinoma, the other a papillomatous adenoma containing large colloid cysts. H. & E. × 21.

FIG. 2.—Thyroid cancer, used for transplantation. G. Alveolar structure replaced in parts by solid growth of cells varying in size, shape and chromatin content of nuclei. H. & E. × 200.

FIG. 3.—Part of transplant, first generation. AAF. Showing multiplicity of structures. v. Gieson. × 21.

FIG. 4.—Detail from Fig. 3 showing structure of nodular area and mitosis in centre. v. Gieson. × 200.

FIG. 5.—Detail from Fig. 3. Cyst with papillomatous growth. The dark spots within the cysts are macrophages filled with pigment. H. & E. × 21.

FIG. 6.—Detail from a first generation transplant showing disorderly growth suggestive of malignancy. G. H. & E. × 200.

FIG. 7.—Area from slow growing tumour (first generation) showing large cysts. G. H. & E. × 90.

FIG. 8.—Detail from first generation graft showing crowding of epithelial cells and large blood sinuses between trabeculae. G. H. & E. × 400.

FIG. 9.—Second generation graft showing invasion of muscle. G. H. & E. × 90.

FIG. 10.—Five-and-a-half month old graft, second generation. Host treated with methylthiouracil. G. H. & E. × 200.

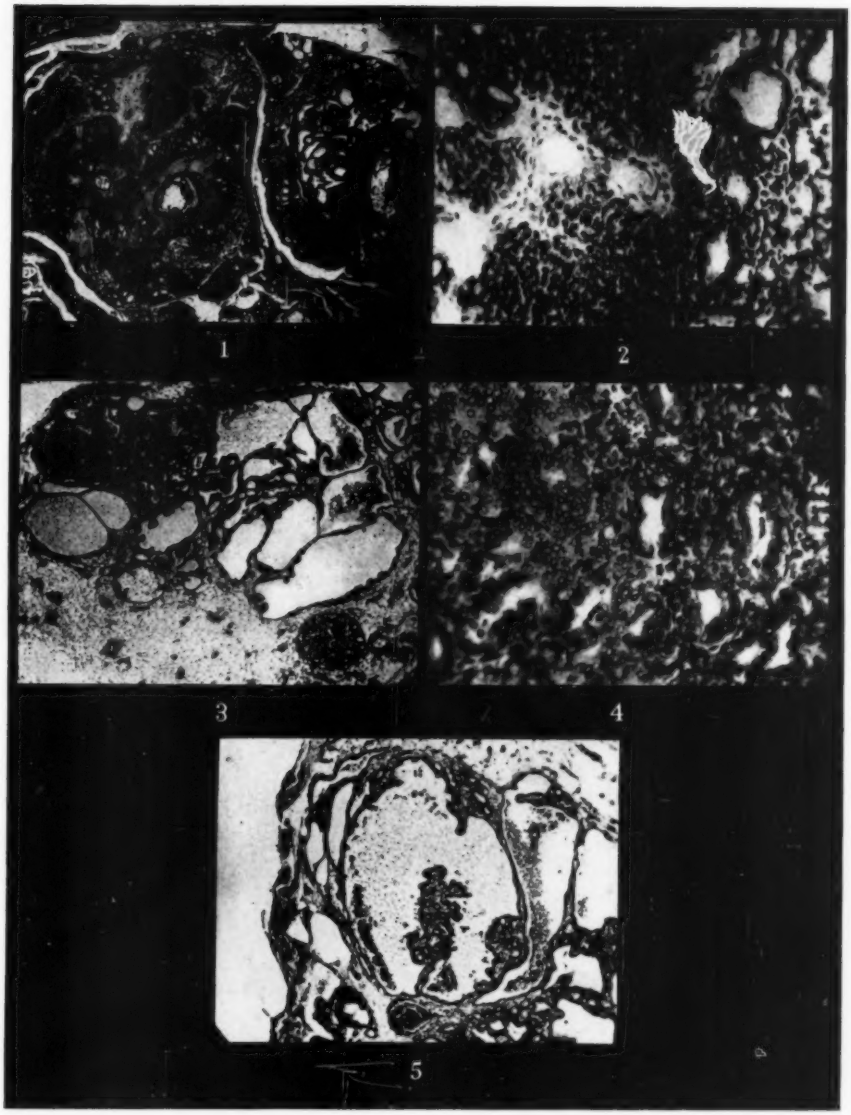
FIG. 11.—Another graft of same origin and age as in Fig. 10. Host completely thyroidectomized. Compare different appearance of epithelium and lack of the large sinuses visible in Fig. 10. G. H. & E. × 200.

FIG. 12.—Rapidly growing graft, first generation, obtained from thyroid tumour in Fig. 2. G. Microfollicular structure. H. & E. × 90.

FIG. 13.—Second generation of the same tumour. G. Note increased density and large size of tumour cells. H. & E. × 400.

FIG. 14.—Second generation graft, 3½ months old. AAF. Large colloid filled acini with low epithelium, in spite of continued administration of methylthiouracil. H. & E. ×21.

NOTE: AAF = Donor received acetamidofluorene + methylthiouracil.
G = Donor received methylthiouracil only.



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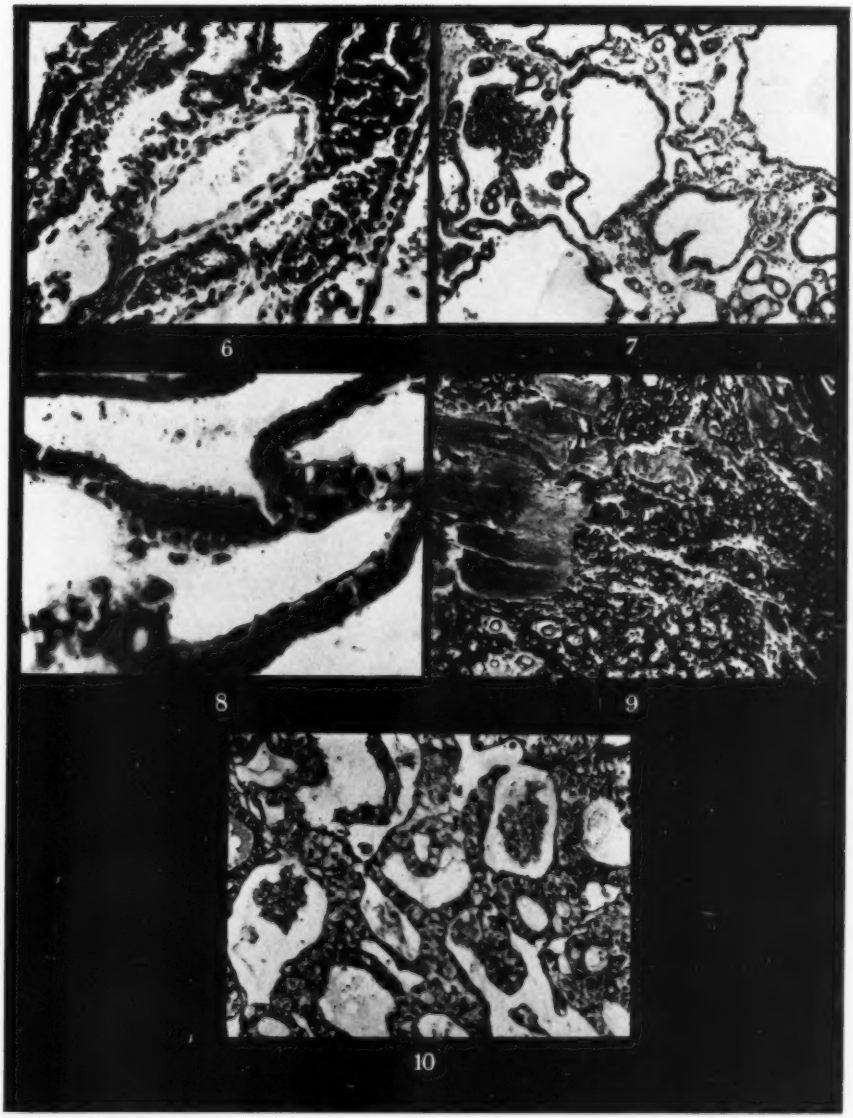

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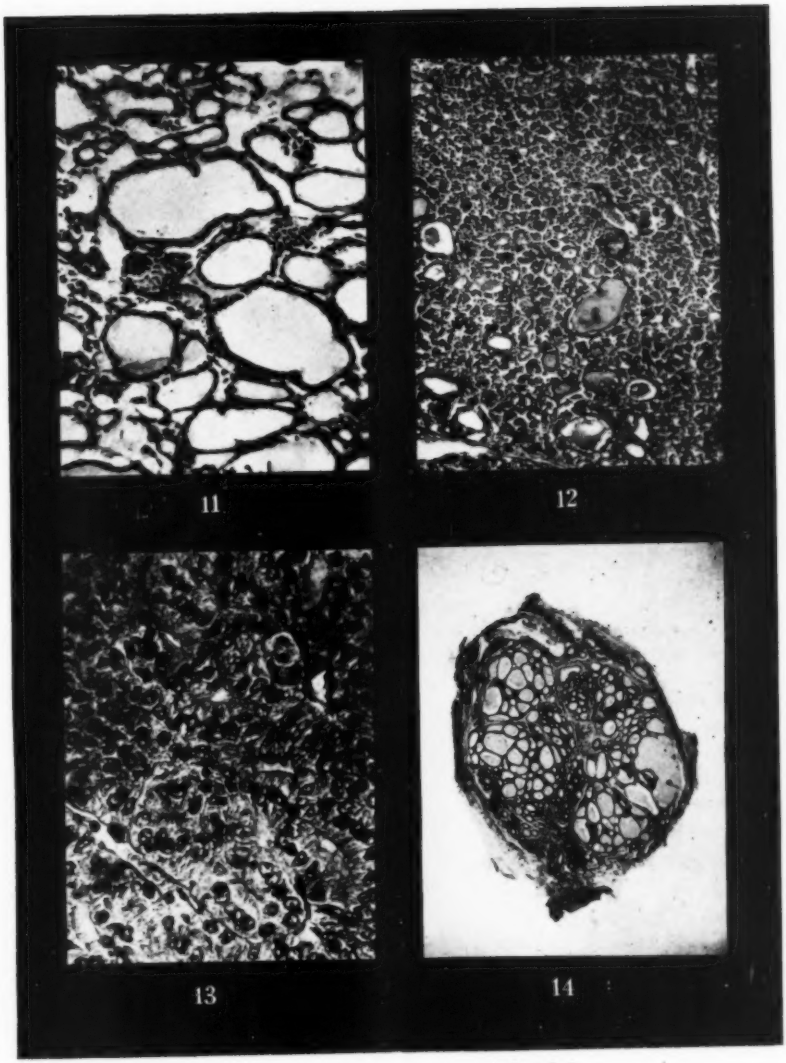

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grafts found in the rat and must be considered a metastasis. This was the only metastasis from a transplant found by naked-eye inspection. In another animal, a tumour cell embolus was seen in a blood vessel leaving the graft. This suggests the spread of transplanted thyroid tumours by the blood stream.

Other observations.

Regression in the size of tumours was observed when the thyroxine deficiency was repaired, but in no case did a palpable tumour disappear. In one animal bearing two actively growing tumours, one of which was removed surgically, the remaining graft was observed for 2½ months after the withdrawal of methylthiouracil. It shrank to about one-half of its maximum size in two weeks and then showed no further change. Histologically this tumour resembled closely the twin tumour removed surgically before methylthiouracil withdrawal. Slight degenerative changes were found.

In no case could the death of an animal be attributed to the presence of a tumour. Those animals with the largest tumours observed showed no signs of cachexia, and their general condition was similar to the non-tumour-bearing controls.

DISCUSSION.

The results reported show a limited transplantability of the thyroid tumours so far obtained. Irrespective of whether the original tumour was induced by methylthiouracil alone or in combination with acetamidofluorene, or whether it had metastasized, no success was achieved with transplants in normal rats. The constant failure of these tumours to take in normal animals, despite the presence of all the signs characteristic of malignancy, shows that the tumours cannot be considered to be autonomous. The inoculated material grows only when a state of thyroid deficiency with its consequent increased output of thyrotropic hormone is maintained in the host. These are the same conditions which were responsible for the production of the original tumour. The necessity for a similar type of hormonal imbalance for the induction and transplantation of other endocrine tumours has been described. Investigations of the interstitial cell tumours of mice have shown that these neoplasms depend for their induction and transplantation on the continued presence of high levels of oestrogen (for references see Hooker, 1948). Abnormal amounts of the same hormone were found to be essential for the transplantation as well as the induction of chromophobe adenomata of the mouse pituitary (Gardner, 1948).

The differences in growth rate of the transplants may be attributed in part to the inoculation of tumour tissues with varying growth potentialities and in part to genetic differences in the host. Those grafts which did not reach weights much above 200 mg. were always encapsulated with dense connective tissue containing numerous round cells. Where capsule formation did not restrict the blood supply, grafts in animals treated with methylthiouracil were larger than those in completely thyroidectomized animals.

Salmon and Severinghaus (1936) and Ingle and Cragg (1939) have shown that normal thyroid tissue can be transplanted into rats with a thyroxine deficiency, so that transplantability alone cannot be considered a criterion of malignancy. The classification of the tumours as malignant is based on the

following evidence : (*a*) invasive growth and ability to metastasize, (*b*) the close resemblance, histologically, to malignant neoplasms of the human thyroid, in particular to malignant adenoma. The fact that the size reached by the grafts is many times greater than the maximum described for hyperplastic rat thyroids shows the neoplastic nature of the transplanted tissue, although it is not evidence for malignancy.

The experimental rat tumours, like many human thyroid tumours however, show only a low grade of malignancy. The discrepancy between clinical and histological criteria of malignancy of human thyroid tumours, which has been discussed recently at a meeting of the American Association for the Study of Goiter (1947), appertains also to the evaluation of experimental thyroid tumours of the rat.

SUMMARY.

1. The behaviour of thyroid tumours induced in rats by methylthiouracil alone or in combination with acetamidofluorene has been studied by homotransplantation.

2. These thyroid tumours were successfully transplanted into rats with a thyroxine deficiency, but not into normal animals.

3. There was no difference in the transplantability of " goitrogen " and " carcinogen induced " tumours.

4. The incidence of takes was 63 per cent in the first generation. In the second generation, 77 per cent of takes were obtained in rats treated with methylthiouracil and 50 per cent in thyroidectomized animals.

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THE ROLE OF THYROXINE DEFICIENCY IN THE FORMATION OF EXPERIMENTAL TUMOURS OF THE THYROID.

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RECENTLY Fitzhugh and Nelson (1948) reported the induction of hepatomas in rats by administration of thiourea or thioacetamide. Fourteen of 29 animals fed for 2 years on diets containing 0·1 per cent or less of thiourea were found to have tumours of the liver. The strain of rats used had an incidence of about 1 per cent of spontaneous hepatic neoplasms. Fitzhugh and Nelson therefore considered thiourea to be a carcinogen. Most workers (Purves and Griesbach, 1946; Money and Rawson, 1947), studying the effects of prolonged administration of thiourea or related compounds on the thyroid have assumed that the tumours developing in this gland were the result of continued stimulation by the thyrotropic hormone and were not due to a direct carcinogenic action of the goitrogen on the epithelial cells of the thyroid. This conception was questioned by Fitzhugh and Nelson. Therefore it seems justifiable to describe shortly the results of an experiment in which the hormonal imbalance essential for tumour formation in the thyroid of rats was induced without taking recourse to the administration of a goitrogen.

MATERIALS AND METHODS.

Five male rats 5–6 weeks old (Group A) received a diet composed of whole meal flour and skimmed milk, to which acetylaminofluorene (A.A.F.) was added in amounts to provide 4 mg. daily for each animal. During the 8th week of the experiment the rats were partially thyroidectomized, and subsequent to the operation they received 2 mg. of A.A.F. daily for 5 weeks. Five female rats (Group B) received a similar diet and a daily dose of 4 mg. of the carcinogen for 25 weeks. These animals were partially thyroidectomized during the 6th week of the experiment. The administration of the carcinogen was withheld from all animals for 48 hours following the operation, which consisted of the removal of the two main lobes of the gland, leaving the isthmus intact. The rats were killed 18–29 weeks later. The larynx and the adjoining part of the trachea as well as the tissues surrounding them were removed *in toto*, fixed in Zenker's solution, and cut in serial sections.

RESULTS.

Only the changes found in the thyroid will be described in this paper.

In two rats of Group A no thyroid tissue was found on histological examination. The other three animals had a hyperplastic isthmus which contained nodules easily distinguishable from the rest of the thyroid tissue. The earliest neoplastic lesion, depicted in Fig. 1, was found in a rat killed 18 weeks after partial thyroidectomy. It consisted of a group of follicles, some of which were dilated and filled with colloid, while others were characterized by closely packed cells with

basophilic cytoplasm. The remainder of the thyroid showed the usual signs of increased functional activity, the follicles containing reduced amounts of colloid and the cells lining them being taller than normal. In a rat killed one week later the isthmus was found to be thickened and dark red in colour. On histological examination an unusual lesion was found to be present in the hyperplastic thyroid tissue (Fig. 2). Around a centrally placed large blood vessel cylindrical thyroid cells with basophil cytoplasm were arranged in a single or in multiple layers. Between the endothelial lining of the central sinus and the atypical thyroid cells, colloid-like material had accumulated. In the section depicted in Fig. 2 these thyroid cells form only two acini; in other sections, however, many acini were seen. The third animal of this group killed 29 weeks after the operation, had a typical adenoma of the thyroid indistinguishable from the nodules found in rats receiving methylthiouracil.

In two rats of Group B, 32 and 37 weeks of age, the residual thyroid tissue contained, apart from similar lesions as described above (Fig. 3), nodules of a different kind. Here microfollicular structures appeared and in some areas densely crowded cells were present, growing in disorderly fashion. These cells were rather small. Their nuclei, rich in chromatin, were surrounded by a small rim of cytoplasm, making it impossible to distinguish cell borders (Fig. 4). These animals were killed 3 and 8 weeks after withdrawal of the carcinogen.

DISCUSSION.

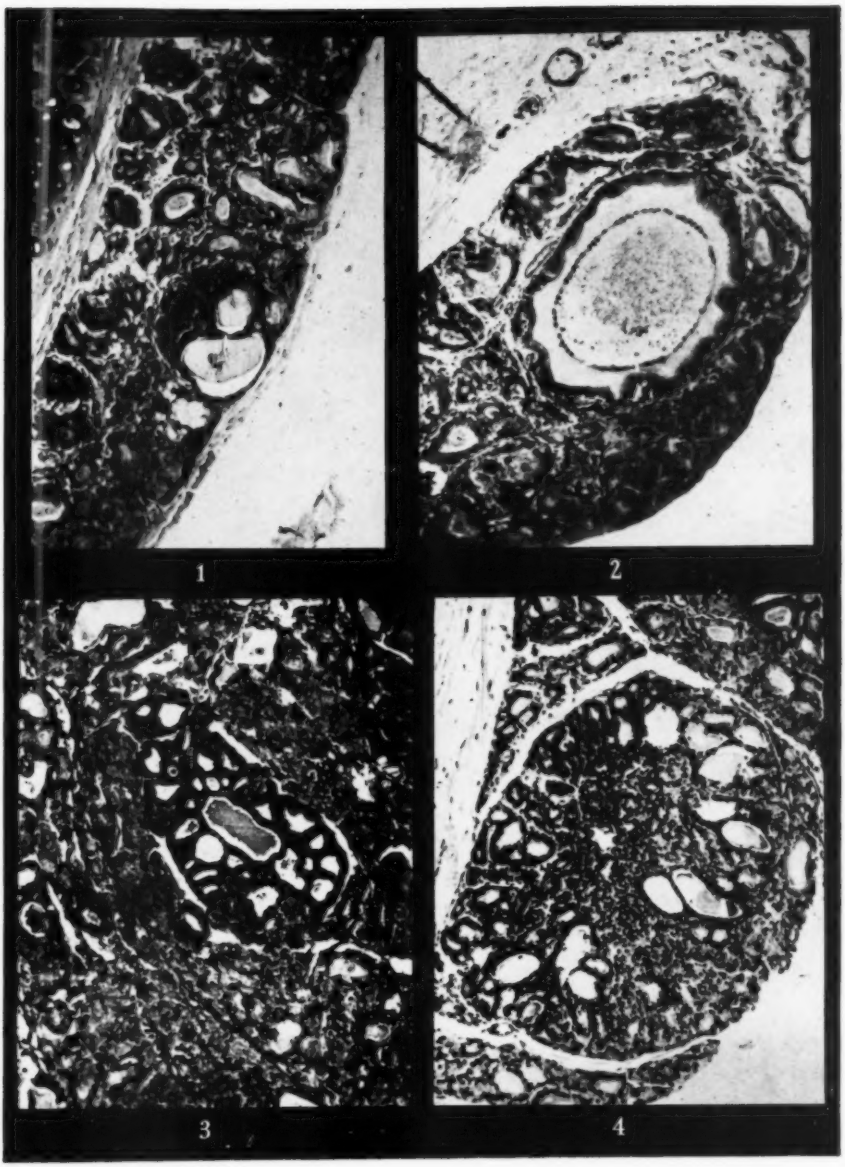
Careful study of the thyroids of many rats receiving A.A.F. has failed to reveal the presence of neoplastic lesions in the thyroid, except when this organ was stimulated by a goitrogen. Not a single thyroid tumour has been found in our material of several hundred rats treated with A.A.F. alone for periods up to 30 weeks. When sacrificed at the age of 9–11 months most of these animals had cancers of the liver, breast gland or other organs. Wilson, DeEds and Cox (1947) observed in two instances nodular grouping of follicles in the thyroids of rats treated with 2-aminofluorene. Their animals were considerably older.

The failure to find neoplastic changes in the thyroid of our rats treated with A.A.F. does not mean that this gland is not susceptible to the action of A.A.F. It has been shown that this carcinogen creates in the thyroid neoplastic cells which remain latent for long periods until the gland is stimulated (Hall, 1948). Tumour formation in the thyroid proceeds very rapidly when A.A.F. acts on a stimulated thyroid (Bielschowsky, 1944; Paschkis, Cantarow and Stasney, 1948). For this reason in the experiment under discussion the administration of A.A.F. was continued for 5 weeks (Group A) and 19 weeks (Group B) after partial thyroidectomy. Single adenomata were found in the former and multiple tumours, some of which already showed anaplastic changes, in the latter group.

From the findings reported in this paper it appears immaterial whether the stimulation is brought about by a goitrogen or by partial thyroidectomy. Both

EXPLANATION OF PLATE.

FIG. 1.—Nodule comprised of acini lined by cells with basophilic cytoplasm. × 114.
FIG. 2.—Tall atypical thyroid cells arranged around sinus. × 114.
FIG. 3.—Hyperplasia of residual thyroid tissue containing a small adenoma. × 114.
FIG. 4.—Anaplastic changes in adenoma of isthmus. × 114.



Bielschowsky.

procedures create a thyroxine deficiency to which the pituitary responds by increasing its output of thyrotropic hormone. These results are in good agreement with those obtained in experiments designed to study the transplantability of experimental thyroid tumours of the rat. Such neoplasms could only be transplanted into animals in which a thyroxine deficiency had been induced chemically or surgically.

The unique observation of Fitzhugh and Nelson (1948) that thiourea produces in one strain of rats first damage and hypertrophy of the liver and finally hepatomas does not invalidate the conception that stimulation by the thyrotropic hormone is the essential factor in the pathogenesis of the experimental tumours of the rat thyroid. Dunhill (1931) believed that "a physiological stimulus" was essential for the induction of human thyroid tumours. In the rat this physiological stimulus has been shown to be an excessive amount of thyrotropic hormone.

SUMMARY.

The induction of adenomata of the thyroid by feeding A.A.F. for 13–25 weeks to partially thyroidectomized rats is reported. No thyroid tumours have been found in rats with intact thyroids treated with A.A.F. for similar periods. The significance of these results for the interpretation of the mechanism responsible for tumour development in the thyroid of rats has been discussed.

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INVESTIGATIONS INTO THE VARYING MANIFESTATIONS OF LEUKAEMIC LESIONS FOLLOWING INJECTIONS OF 9:10-DIMETHYL-1:2-BENZANTHRACENE INTO DIFFERENT SUBCUTANEOUS SITES IN STREET MICE.

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IN an earlier paper (Rask-Nielsen, 1948) it was reported that, besides local tumours, leukaemia developed in about 20 per cent of mice following injection into various organs or the subcutaneous tissue of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene. This development of leukaemia, with an incidence 10 to 20 times greater than that of spontaneous leukaemia in the strain concerned, and manifest at a considerably earlier age than spontaneous cases of the disease, appeared as isolated thymic hyperplasia, generalized leukaemia with or without

thymic tumour or, in a few cases, lymphosarcomas at other sites than the thymus. Moreover, the ratio between thymic hyperplasia and generalized leukaemia differed considerably with the site of application, and it was especially emphasized that this variance was found even after injections of the hydrocarbon into two different subcutaneous sites, namely, into the left flank and below the second lowest right mammary papilla. The latter observation in particular seemed rather incomprehensible, and as similar results have not been reported by others it was found advisable to add some more experiments to those already performed. This paper gives a report on these experiments.

TECHNIQUE AND MATERIAL.

Street mice have been used for the experiments. The spontaneous incidence of leukaemia in this strain is 1 to 2 per cent (Rask-Nielsen, 1948; Lefèvre, 1945). At the age of 5 to 7 weeks all the animals were injected with 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene suspended in a mixture of solid and liquid paraffin. A volume of 0·01 c.c. contained 0·5 mg. hydrocarbon (Rask-Nielsen, 1948). In the three groups included in the experiments the injection was administered subcutaneously into the left flank, the interscapular region, and below the second lowest right nipple, respectively. Some of the last mentioned experiments, originally made for other purposes, were carried out on female mice exclusively; equal numbers of male and female mice were used in the rest of the experiments. The three experimental groups included 6, 4 and 7 separate experiments, respectively, performed within a period of about two years. The animals were fed on whole wheat and rolled oats with the addition of cod liver oil and yeast once a week. The supply of drinking water was unrestricted. All animals were autopsied. Lungs, liver, spleen and kidneys from animals macroscopically suspect for leukaemia were examined microscopically; also the thymus and lymph nodes when found enlarged.

EXPERIMENTS.

Survival time.

The numbers and survival time of the animals are recorded in Table I. As indicated, only very few of the animals attained old age; this is not merely attributable to the fact that a considerable number of the animals died from local tumours or leukaemias, most of them at the age of from 4 to 6 months, but is as much due to the fact that many of the animals succumbed to the treatment, since 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene given subcutaneously must be considered close on the lethal dose for Street mice.

Incidence of tumours.

In 66, or about 10 per cent, of the mice, spindle cell sarcomas and a few squamous cell carcinomas developed at the site of injection; they will be dealt with in a later paper. Moreover, a great number of leukaemias developed. The total number of leukaemias within the three experimental groups is recorded in Table II, where the percentages of leukaemias are also stated, calculated on the basis of the effective number of animals, which is the number of animals attaining a survival time of at least four months, since the youngest leukaemic animals in the three groups died at that age. According to Table II the total

TABLE I.—*Numbers and Survival Time of the Experimental Mice.*

Site of injection.	3 months.		6 months.		9 months.		12 months.		15 months.		18 months.		21 months.	
	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.	♂.	♀.
Flank	138	168	69	83	16	28	8	12	2	0	1	0	0	0
Interscapular region	114	119	62	69	18	30	8	19	2	12	0	3	0	0
Mammary region	87	258	49	115	13	27	3	9	1	1	1	0	0	0

TABLE II.—*Incidence of Leukaemia.*

Site of injection.	Male mice.							Female mice.								
	Effective total.	Total leukaemia.		Thymic tumours.		Generalized leukaemia.		Effective total.	Total leukaemia.		Thymic tumours.		Generalized leukaemia.		Other types of leukaemia.	
		Number.	%.	Number.	%.	Number.	%.		Number	%.	Number.	%.	Number.	%.	Number.	%.
Flank	98	14	14·3	6	6·1	8	8·2	124	26	21	16	13	7	5·6	3	2·4
Interscapular region	91	12	13·2	8	8·8	4	4·4	94	22	23·4	3	3·2	17	18·1	2	2·1
Mammary region	69	8	11·6	2	2·9	6	8·7	185	49	26·5	8	4·3	37	20	4	2·2

TABLE III.—*Minimum, Maximum and Average Latent Period (in Weeks).*

Site of injection.		Thymic tumours.				Generalized leukaemia.			
		Number of cases.	Latent period.			Number of cases.	Latent period.		
			Minimum.	Maximum.	Average.		Minimum.	Maximum.	Average.
Flank	Males	6	11	48	24·1	8	13	34	20·2
	Females	16	12	50	23·3	7	15	41	21
Interscapular region	Males	8	14	30	22·6	4	14	30	20·7
	Females	3	19	29	25	17	9	55	19
Mammary region	Males	2	21	..	21	6	11	31	20·5
	Females	8	12	57	23·9	37	9	42	18·9

incidence of leukaemia after injection of the hydrocarbon in the three different sites has been identical in male animals (11·6 to 14·3 per cent) and identical in female animals (21 to 26·5 per cent) but the incidence in female animals has been significantly greater than in male animals.

Table II also indicates that the development of leukaemia in the males of the three groups is apportioned by isolated thymic tumours in 2·9 to 8·8 per cent and in 4·4 to 8·7 per cent by generalized leukaemias, including cases with enlarged lymph nodes, generally without, but, in about a third of the cases, combined with a minor thymic tumour. Within every group, and with no difference between the three groups, about equal numbers of isolated thymic tumours and generalized leukaemias have been observed in the male animals. No other leukaemic manifestations have been observed in the males.

Following injection into the flank isolated thymic tumours developed, however, in 13 per cent, and generalized leukaemias in 5·6 per cent of the female mice; following interscapular injection thymic tumours were found in 3·2 per cent, and generalized leukaemias in 18·1 per cent of the females. Following injection into the mammary region thymic tumours were found in 4·3 per cent, and generalized leukaemias in 20 per cent of the females. About 2 per cent, totalling 9 cases, of other types of leukaemias were found uniformly distributed in all three experimental groups. These were 3 cases of enlarged liver and spleen with no other leukaemic lesions, and 6 cases of lymphosarcoma involving the lumbar muscles. The latter lesion was also found in 16 female mice suffering from generalized leukaemia, of which 12 were injected in the mammary region. A pronounced increase in the numbers of thymic tumours following injection into the flank, and of generalized leukaemia following injections into the other two regions has thus been found in the female animals.

With the exception of one case of plasma cell leukaemia all the leukaemias have been stem cell leukaemias.

It should be emphasized that the incidence of all cases of leukaemia as well as the incidence of thymic tumour and of generalized leukaemia differed to some extent in the 6, 4 and 7 separate experiments performed in the three groups. Probably, a concurrent cause of this may be found in the effect on the general condition of the animals of the carcinogenic treatment since the incidence of leukaemia seemed to be greater in experiments where 50 to 75 per cent of the injected animals survived at the time of death of the first leukaemic animal, whereas higher as well as lower mortality coincided with a lower incidence of leukaemia.

Latent period.

A survey on the minimum, maximum, and average latent period of the development of leukaemia is recorded in Table III, indicated by the number of weeks from the time of injection to the death of the animals. It will be observed that the latent period was a little shorter in cases of generalized leukaemia (18·9 to 21 weeks) as compared to that of the cases of thymic tumours (21 to 24·1 weeks), and that the latent periods in each of the two types of leukaemia was identical in male and female animals, and in all three experimental groups.

This observation indicates that one type of leukaemia has not, by forestalling the other type of leukaemia by its more rapid development, been able to reduce the number of animals prone to development of the other type of leukaemia.

It may be added that the average latent period of local tumour development, sarcomas and carcinomas in 66, or 10 per cent, of the animals, in the three experimental groups has been 19·7 to 22·4 weeks, which is almost identical to the latent period of leukaemia, thus indicating that the incidence of local tumours has hardly been able to influence the number of leukaemias observed. It is, however, remarkable that out of those 66 cases of local carcinomas and sarcomas only four, or 3 per cent, were found among the 131 leukaemic animals, whereas the remaining 62 local tumours were found among the 530 non-leukaemic animals, giving an incidence of 11·7 per cent in the non-leukaemic mice.

Leaving apart animals proving refractory to the carcinogenic influence, the experiments show that some mice reacted by developing local sarcomas and carcinomas, some by developing leukaemia. This is difficult to explain. The latent period being equal in the two types of neoplasia it can hardly be assumed, as already mentioned, that development of one type has interfered with the development of the other by way of forestalling it. There may be an indication that the phenomenon referred to in cancer literature, of one type of tumour being able to " immunize " against other types, gives some kind of explanation to this. On the other hand, genetic differences in the experimental animals may be the deciding cause. This can only be proved through investigations on the offspring of the tumour-bearing animals, but so far such investigations have not been possible in the experiments reported in this paper.

DISCUSSION.

It has been shown that the subcutaneous injection of 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene has caused the development of leukaemia in no less than 20 per cent of the mice. This is due to the specific properties of that particular hydrocarbon since subcutaneous injection of a similar dose of the hydrocarbons most commonly used, benzpyrene, 1:2:5:6-dibenzanthracene and methylcholanthrene, in Street mice under identical experimental conditions induced spindle cell sarcomas almost exclusively (Rask-Nielsen, 1948). As it must be taken for granted that remote tumour development is caused by the local influence of hydrocarbon absorbed from the site of application, it might be assumed that the leukaemogenic effect of 9:10-dimethyl-1:2-benzanthracene is due to the absorption of this particular hydrocarbon from the subcutaneous tissue being less impeded than in the case of the three other hydrocarbons dealt with. It might even be presumed that the difference in the manifestations of the leukaemic lesions is in some way or other connected with the absorption rate of the hydrocarbon. The following experiment will probably justify this presumption: 0·5 mg. of 9:10-dimethyl-1:2-benzanthracene was injected into female mice, in one series into the left flank, and in a second series into the mammary tissue. At regular intervals of two weeks mice in both series were killed; the skin covering the site of application was pulled away, and by fluorescence in ultraviolet light it was estimated whether some hydrocarbon remained at the site of application. From three to eight weeks after injection fluorescence was found in mice from both series; in the series of mice injected into the mammary tissue seven surviving animals showed no signs of fluorescence 10 weeks after the injection, whereas fluorescence remained 10 weeks after injection in four out of eight mice injected into the flank; fluorescence also remained in one out of two animals 12, 14 and 16 weeks after injection into the flank. If any deduction can be

drawn from such small numbers it seems as if the hydrocarbon is more rapidly absorbed following injection into the mammary tissue than is the case when subcutaneous injections have been made into the flank. This is probably due to a higher lipoid content in the former than in the latter tissue of female mice. Since localization of the leukaemic lesions is different following injections into these two sites this observation indicates that the manifestation of leukaemia is influenced by the quantity of hydrocarbon absorbed, and perhaps also by the absorption rate which would determine the maximum concentration of the hydrocarbon obtained in the body. It is also probable that differences in the rate of elimination or in the transformation into non-carcinogenic metabolites may have been of importance.

It should be noted here that variation in the manifestations of leukaemia in relation to the site of application of the hydrocarbon was observed in female mice exclusively. This may be due to the fact that the differences in the rates of absorption of the hydrocarbon, dependent on the localization of the site of application, are presumably more pronounced in females than in males because the subcutaneous tissue in the mammary region in female mice has a higher lipoid content than that in male mice, whereas in the subcutaneous tissue of the flank the lipoid content must be considered to be equal in males and females. It cannot be denied that other conditions may have interfered, such as the presence of female sex-hormones. No correlation between the development of local sarcomas and leukaemia has been observed, probably because the amount of hydrocarbon administered was so large that, although the amount absorbed varied, an amount sufficient or even more than sufficient to cause development of local tumours still remained at the site of application.

Whereas spontaneous thymic tumours in Street mice usually occurred in younger animals than did generalized leukaemias (Lefèvre, 1945 ; Rask-Nielsen, 1948) the two types of leukaemic lesions developed almost simultaneously in the experiments dealt with here, and even in a considerably shorter time than did the spontaneous cases. This is in harmony with previous observations on the acceleration of leukaemia in Street mice (Lefèvre, 1945) where the survival time after painting with 9:10-dimethyl-1:2-benzanthracene was identical whether the animals suffered from isolated thymic tumours or from generalized leukaemia, which latter, however, was in some cases presumably associated with thymic tumours. Dlb mice painted with 9:10-dimethyl-1:2-benzanthracene generally developed generalized leukaemia, and only in rare cases thymic tumours (Law, 1941). This apportionment of the leukaemic lesions was also found in dlb and Aka mice painted or subcutaneously injected with 9:10-dimethyl-1:2-benzanthracene (Engelbreth-Holm and Lefèvre, 1941). With different doses Lefèvre found in later experiments that the development of leukaemia in dlb and Aka mice was only accelerated if they had been painted with 9:10-dimethyl-1:2-benzanthracene, and the acceleration would then consist in a relative increase in the numbers of thymic tumours whereas no acceleration occurred when methylcholanthrene was administered. Other experiments with dlb mice (Morton and Mider, 1941) showed that generalized leukaemias predominated, and only a few cases of thymic tumours developed following painting with 0·5 per cent methylcholanthrene and benzpyrene in benzene, but the relative number of thymic tumours increased following painting with 0·25 per cent methylcholanthrene in benzene, or even more, in acetone. More thymic tumours developed in F mice

painted with benzpyrene than was the case after identical treatment with methylcholanthrene (Kirschbaum and Strong, 1942).

Further, it has been observed in dlb mice that comparatively more thymic tumours developed when relatively few paintings were applied, and comparatively more generalized leukaemias when more paintings were applied. A considerable number of mediastinal lymphosarcomas developed in various strains after treatment with oestrogens but in some cases these were associated with generalized leukaemia (Gardner, Dougherty and Williams, 1944). Finally, it should be mentioned that the increase in number was considerably greater in thymic tumours than in generalized leukaemias following general X-ray irradiation, particularly in two out of the three strains examined. The latent period of these thymic tumours was shorter than that of the generalized leukaemias (Furth and Furth, 1936). The latter finding is in accordance with the experiments of Kaplan (1947), who found an increase in the number of thymic tumours following general X-ray irradiation of A mice ; Lorenz and his associates found that the earliest lymphosarcomas developing in hybrid mice constantly exposed to gamma radiation were predominantly mediastinal, whereas those developing later tended to involve the spleen and lymph nodes more frequently (Kaplan, 1948). Thus, it might be assumed that the genetic constitution of the animals, the kind of leukaemogenic agent, the dose administered and the vehicle employed have all exerted a certain influence not only on the latent period but also on the localization of the leukaemic manifestations.

In a previous paper (Rask-Nielsen, 1948) certain observations have been reported indicating that the thymus is the primary site of development of leukaemic cells in Street mice, as is also the case in strains AK (McEndy, Boon and Furth, 1944), A and C_{57} (Kaplan, 1947, 1948), other organs being invaded secondarily from the thymus. Provided that this observation holds good the effect exerted by factors determining the manifestation of the leukaemic lesions probably consists in their ability to determine whether leukaemic cells remain as lymphosarcomas in the thymus or disseminate into other organs, or in their ability to modify the resistance of the organism to dissemination of the leukaemic cells.

Such a difference in resistance to dissemination of leukaemic cells has been observed by Hogreffe (1947), who found that intraperitoneal transplantation of leukaemic cells to mice of the same strain (AKA) and to the first filial generation of the latter and a non-leukaemic strain (B), and to mice from back-crossing F_1 to AKA mice caused development of typical generalized leukaemia, whereas mice from back-crossing F_1 to B mice and mice from the second filial generation developed lymphosarcomas at the site of injection. Measurements of cells showed besides " that in some cases the nuclei were found on an average to be larger in the localized forms of the takes than in the corresponding generalized, indicating a lesser degree of differentiation in the former."

In the experiments reported in this paper there may have been certain differences in the degree of differentiation of the cells of the two types of leukaemic lesions, thymic hyperplasia and generalized leukaemia. Provided this has been the case the factor or factors, so far unknown, determining which type develops must be presumed to have been able to exert some influence on the stage of differentiation at which normal cells are converted into malignant leukaemic cells.

As to the cause of the rare occurrence of coincidence of local tumour and leukaemia nothing can be elucidated. Skin tumours as well as leukaemias were observed in several Street, AKA and dlb mice painted by the method described by Murphy and Sturm (1925) with 9:10-dimethyl-1:2-benzanthracene or methylcholanthrene (Engelbreth-Holm and Lefèvre, 1941 ; Lefèvre, 1945). In most of the reported experiments on acceleration the same observation has been made. Kirschbaum and Strong (1942) observed, however, some antagonism between the two types of tumour and found skin tumours in 93 per cent of the non-leukaemic F mice painted with methylcholanthrene as compared with 19 per cent of the leukaemic mice. In mice from several strains painted with methylcholanthrene Morton and Mider (1941) observed that lung tumours and leukaemias did not occur simultaneously and, moreover, that skin tumours together with lung tumours, or skin tumours combined with leukaemias, developed but rarely.

SUMMARY.

Following injection into Street mice of 0·5 mg. 9:10-dimethyl-1:2-benzanthracene subcutaneously into the left flank, interscapularly and below the second lowest right mammary papilla, leukaemia developed in 11·6 to 14·3 per cent of the males comprising almost equal numbers of isolated thymic tumours and generalized leukaemias. Leukaemia developed in 21 to 26·5 per cent of the female mice ; an increased number of thymic tumours (13 per cent) was observed after injection into the flank, whereas generalized leukaemia developed in 18·1 and 20 per cent following interscapular injection and injection into the mammary region, respectively.

Local spindle cell sarcomas developed in about 10 per cent of all the animals. The average latent period of local tumours, thymic tumours and generalized leukaemias was identical, i.e. 19 to 24 weeks.

The findings are discussed.

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EFFECT OF CHOLINE ON EXPERIMENTAL CARCINOGENESIS.

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THE investigation now recorded was prompted by the remarkable findings of Copeland and Salmon (1946), who observed a high incidence of tumours in rats fed on a diet free from choline, but in other respects adequate. These workers fed a series of 24 rats with a diet in which the protein consisted of 6 per cent of casein and 30 per cent of peanut meal which had been extracted with alcohol. The 14 animals which survived for 8 months and longer developed a variety of tumours of different organs (liver, lung, etc.). Nineteen control rats fed on the same diet supplemented with 20 mg. of choline per rat per day failed to show any abnormalities. Corroboration of these results appeared to be afforded by Staub, Viollier and Werthemann (1948), who observed multiple liver adenomas in rats kept on a choline-deficient diet for 3 to 4 months. The implication that the tumours were the result of absence or deficiency of choline clearly merited further investigation. A possible interpretation is that choline antagonizes the action of some unknown intrinsic carcinogenic factor.

Choline deficiency as a cause of the tumours seems difficult to reconcile with the evidence from choline estimations in tissues by Jacobi, Baumann and Meek (1941), whose results indicated that rats are able to synthesize choline. On the other hand, it is of interest that Ritchey, Wicks and Tatum (1947) found that tumour tissues had a greatly increased choline content (of the order of 250 per cent).

The effect of choline on tumour growth had previously been examined by Jacobi and Baumann (1942), who studied epithelial tumours induced in mice by 20-methylcholanthrene and also the Flexner-Jobling carcinoma of the rat. They observed no effect on the growth of tumours or on the survival time of the animals, neither with a diet deficient in choline nor in a diet supplemented by excess of choline. These experiments did not exclude the possibility that choline, although without action on established tumours, may prevent or delay their appearance if administered from the beginning of application of the carcinogen.

To test this possibility we carried out the following experiment: Forty-seven young white rats (8 litters) obtained from a dealer were divided into two groups. Each group consisted of similar numbers of litter mates from each litter, and contained approximately equal numbers of males and females, which were kept in separate cages. The diet consisted of rat cakes (supplied by the North-Eastern Agricultural Co-operative Society, Ltd., Aberdeen), supplemented once a week by cod-liver oil, with water *ad lib.* One group of rats was given 1 per cent of choline hydrochloride in the water from the day of application of the carcinogen.

Each of the animals in both groups was injected subcutaneously with 2·5 mg. of 1:2:5:6-dibenzanthracene suspended in 0·5 ml. of tricaprylin. The injections were made into the lower part of the back, care being taken to prevent spreading under the skin or leakage of the oily suspension. The injections were twice repeated, at intervals of about two months.

Survival of the animals was good, in spite of the large doses of choline. The first tumours appeared after about 6 months, almost simultaneously in both groups of rats. After 9 months the experiment was terminated. Ten of the 13 males in the choline-treated series and 9 of the 12 males in the control series then had tumours. The corresponding figures for the females were 5 out of 11 in the choline-treated series and 7 out of 11 in the control series. The lower incidence of tumours in the female rats is of interest, but is, of course, not significant. The surviving male rats were used for estimation of glutathione and ascorbic acid in the livers and tumour tissue by Dr. Meduski, as reported in the following paper (Meduski, 1949).

Some of the animals from each of the four groups showed on *post-mortem* examination changes in the lungs of the type associated with pneumonia, common in rats (Griffith and Farris, 1942). The rats were weighed at monthly intervals and the weights of litter mates compared. Those receiving choline were consistently about 10 per cent below the weight of their control litter mates, and on an average the tumour size was smaller in the choline-treated series. A tumour from a choline-treated male rat was transplanted into 4 young albino rats (aged 5 weeks) and grew in 3 of them, confirming the malignancy of the tumours.

It may be concluded from these results that large doses of choline had no effect on the induction of tumours in rats by 1:2:5:6-dibenzanthracene.

We are greatly indebted to Professor S. Alstead, Department of Materia Medica, for placing at our disposal the facilities of his animal house. We thank the British Empire Cancer Campaign for a grant which has supported this work, and we also thank Misses E. B. Duff and E. I. Brander for their excellent care of the animals.

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THE ASCORBIC ACID AND GLUTATHIONE CONTENT OF THE LIVERS OF RATS WITH SARCOMATA INDUCED BY 1:2:5:6-DIBENZANTHRACENE.

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THE ascorbic acid and glutathione content of the liver during the early stages of cancer production with hydrocarbons has been the subject of several investigations. In 1938 Boyland and Mawson found that 3:4:5:6-dibenzcarbazole in doses of 0·25–0·5 mg. per 20 g. mouse produced a rise in glutathione content which was maximal during the first 20 days after injection. No change was observed after 2 mg. 1:2:5:6-dibenzanthracene or 1 mg. methyl-cholanthrene. None of these compounds induced any change in the ascorbic acid content of the liver. Kennaway, Kennaway and Warren (1944*a*) concluded from observations on several carcinogenic and non-carcinogenic hydrocarbons that the former increase the ascorbic acid content of the liver of mice whereas the latter do not. There were no changes in glutathione content. The interval between the injection of the hydrocarbon and the examination of the liver was short, in most instances less than 20 days. In a later paper Elson, Kennaway and Tipler (1949) give data of experiments in which 1:2:5:6-dibenzanthracene (500 mg. per kg., intraperitoneally) was given to rats on diets with different protein contents. They observed an increase in liver ascorbic acid in each group during the first three weeks.

The above experiments were carried out during the early period after the administration of the carcinogenic hydrocarbon. Recently an opportunity arose of examining the ascorbic acid and glutathione contents of livers from rats bearing sarcomata as a result of 1:2:5:6-dibenzanthracene administered some nine months before. These data show that, compared with a control group of rats, the ascorbic acid content of the liver is unchanged, but the glutathione content is significantly raised.

The animals were male albino rats which had been injected with 1:2:5:6-dibenzanthracene and fed on adequate diets supplemented in some cases with choline hydrochloride. Details of this treatment are given in the preceding paper (Cook and Schoental, 1949). Nine months after the beginning of the injections the rats were given to me by Professor Cook and Dr. Schoental. A control series of rats were kept under the same conditions but were not injected. Some of these animals were given choline in their drinking water for one week before slaughter.

The rats were fasted 18 hours, and then killed by a blow on the head, and the liver was rapidly removed and also the tumour was dissected out. These were at once taken to the cold room, weighed, ground with sand and freshly prepared 5 per cent metaphosphoric acid. From this stage onwards the ascorbic acid determination was carried out with 0·010 per cent 2:6-dichlorophenol-indophenol according to the method of Kennaway, Kennaway and Warren (1944*b*).

Glutathione was estimated by Leaf and Neuberger's (1947) adaptation of Fujita and Numata's (1938) iodometric procedure, using a final concentration of 0·5 per cent KI in the titrated fluid. The usual reagent blanks were carried out. Experiments with pure glutathione (B.D.H.) gave 95–98 per cent recovery with this method.

All chemical procedures were carried out in the cold room at 0° C. Three to five estimations were carried out on each sample and the results were averaged.

RESULTS AND DISCUSSION.

Glutathione content of liver and tumour tissue.

Table I shows that the control group of rats had the same glutathione content of liver, whether fed choline in the drinking water or not. The average figure

TABLE I.—*Concentrations of Glutathione and Ascorbic Acid in Liver and Tumour Tissue (Fresh Weight).*

Group of rats.	No. in group.	Mean body weight (g.).	Liver.		Tumour.	
			Glutathione (mg. per g.).	Ascorbic acid (μg. per g.).	Glutathione (mg. per g.).	Ascorbic acid (μg. per g.).
Controls—						
(*a*) Stock diet	2	218	1·56	163	—	—
(*b*) Stock diet and choline	3	183	1·65	116	—	—
All controls	5	—	1·61*	—	—	—
Injected—						
(*a*) Stock diet	4	264	1·85	164	0·43	112
(*b*) Stock diet and choline	7	201	1·89	171	0·61	126
All injected	11	—	1·87*	—	—	—

* Difference in glutathione content of livers of injected and control animals = 0·26 mg. per g. $t = 2{\cdot}90$; $P = 0{\cdot}02 - 0{\cdot}01$ (significant).

for these two groups is 1·61 mg. per g. fresh liver, and is in good agreement with the values obtained by other workers for rats subsisting on stock diets. Thus Thompson and Voegtlin (1926) obtained an average value of 1·77 mg. per g. liver for albino rats of weight 188–206 g. and starved 18 hours; they used Tunnicliffe's (1925) iodometric method, in which nitroprusside is employed as an external indicator. Woodward (1935), using the glyoxalase method, found 1·72 mg. per g. wet tissue in albino rats starved 24 hours. Leaf and Neuberger (1947) obtained a value of 1·60 mg. per g. tissue for albino rats starved for 24 hours. The latter workers used a stock diet closely resembling the one employed in the present series of experiments.

The rats injected with 1:2:5:6-dibenzanthracene had a significantly higher glutathione content in their livers than the control animals; there was no difference in glutathione content of animals receiving and not receiving choline in the drinking water. A raising of glutathione content of the liver is not likely to be due to the existence of the tumour, since it has been observed that glutathione content is lowered when Flexner-Jobling carcinoma, rat sarcoma

(Voegtlin and Thompson, 1926), Walker carcinoma and Philadelphia sarcoma (Woodward, 1935) are transplanted; this depression of glutathione content is thought to be due to inanition (Toennies, 1947). In this connection it may be observed that one of our rats had a large parasitic cyst of the liver, and in this instance the glutathione content was 1·01 mg. per g. liver. The only authors recording a rise in liver glutathione content are Fujiwara, Nakahara and Kishi (1938), who found average values of 2·43 mg. per g. liver with a transplantable hepatoma.

A direct action of 1:2:5:6-dibenzanthracene five months after the last injection can be excluded, since Berenblum and Kendal (1936) have shown for the mouse, and Jones, Dunlap and Gogek (1944) have shown for the rat that this carcinogenic hydrocarbon is fairly rapidly eliminated. This suggests that the raised glutathione content of liver is the result of late general changes resulting from injections of the carcinogen. This may be compared with the failure of other workers, mentioned in the introduction, to obtain changes in liver glutathione concentration shortly after injection of 1:2:5:6-dibenzanthracene.

The observations that choline administration does not affect the glutathione content of liver and tumour tissue is interesting in view of the high phospholipid content and turnover of tumour tissue, especially in the case of the choline-containing lecithins (Jacobi and Baumann, 1942).

Ascorbic acid content.

There is obviously no differences in the ascorbic acid content of the livers of the normal animals not given choline and of the livers of the injected animals, whether given choline or not. The lower ascorbic acid content of the livers of normal animals receiving choline in their drinking water is not significant (P: 0·1–0·05), but might be worthy of further investigation in larger groups of animals.

The ascorbic acid content of the tumour tissue did not differ for rats fed choline and rats not fed choline.

There does not appear to be any relationship between the ascorbic acid content and glutathione content of the different tumours, nor is there any relationship between the ascorbic acid content of the liver and tumour or between glutathione content of the liver and tumour for individual animals.

SUMMARY.

(1) The glutathione and ascorbic acid content of the liver and tumour tissue has been examined in a group of rats five months after the last of three injections of 2·5 mg. 1:2:5:6-dibenzanthracene in 0·5 ml. of tricaprylin given at 2-monthly intervals.

(2) In comparison with a control group of rats maintained on the same diet for an equal length of time the livers of the tumour-bearing rats contained significantly more glutathione, but the ascorbic acid content was not notably different in the two groups. The giving of choline to some of the injected rats did not have any effect on the ascorbic acid or glutathione content of their livers or tumours.

(3) There was no relationship between the ascorbic acid and glutathione content of the tumour tissue and of the liver in individual animals.

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EFFECTS OF REPEATED X-RADIATION OF THE WHOLE BODY ON THE DEVELOPMENT OF TUMOURS IN RATS DUE TO FEEDING *p*-DIMETHYLAMINOAZOBENZENE.

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THE effect of X-radiation in producing malignant growth in man was found soon after X-rays were introduced into general use in medicine. In 1909 eleven cases were reported in England (Rowntree, 1909). The tumours occurred after repeated exposures over a long period and they were preceded by dermatitis. They appeared on the parts of the skin directly exposed to the rays, and it was therefore concluded that the radiation absorbed in superficial layers was responsible for these changes. In animal experiments Bloch (1924) produced epitheliomata of the rabbit's ear with large doses of X-rays only, and X-rays were found to provoke malignant changes when applied to inflamed tissue in rabbits (Lacassagne and Vinzent, 1929; Lacassagne, 1933; Burrows, Mayneord and Roberts, 1937; Burrows and Clarkson, 1943). In combination with other carcinogenic agents, e.g. tar (Castiglioni, 1927) or carcinogenic hydrocarbons (Mayneord and Parsons, 1937), X-rays accelerated the appearance and the growth of tumours in mice. Although in these combinations both agents were acting on the skin, it was considered that the effect of X-rays was partly direct, in stimulating the cells involved in cancer production, and partly indirect, in breaking down the defence mechanism of the body. Russ, Chambers, Scott and Mottram (1919)

concluded as a result of their experiments on the effect of small doses of X-rays on the blood count and on the resistance to transplanted tumours in rats, and from evidence produced by Murphy and Morton (1915) and Murphy and Taylor (1918), that X-rays, apart from their direct action on tissue cells have two indirect actions: "(*a*) large doses of X-rays, by destroying the immune conditions, will favour the growth of tumours, and (*b*) small doses, by producing immune conditions, will help to overcome the tumour."

In order to find out whether irradiation has an indirect effect on the development of tumours, experiments on the combined effect of irradiation and a carcinogenic compound not acting on the same organ seemed of interest. The study of the influence of total body irradiation on the development of hepatic tumours, which occur with great regularity in rats fed with *p*-dimethylaminoazobenzene seemed suitable for this purpose. It was intended to irradiate with small doses, but opinions differ as to the amount of radiation that should be considered small, particularly where it is applied repeatedly over a long period of time, e.g. about half the expected lifetime of the experimental animal. In the present exploratory experiment, both penetrating and soft X-rays were used in doses which appeared reasonably small. The dosages were calculated so that the skin of the back should receive about the same amount of radiation with the soft as with the penetrating rays. The liver, however, which is the site of tumours produced by feeding *p*-dimethylaminoazobenzene, would receive with hard rays about the same amount as the skin, but with soft rays hardly any radiation at all. It was thought that under these conditions a direct and an indirect action of the radiation before and during the establishment of a tumour might be distinguished.

EXPERIMENTAL.

Forty male and 10 female Wistar rats, weighing about 100 g. at the beginning of the experiment, were given a semi-synthetic diet, in which 53 per cent of the calories were supplied by starch and sugar, 17 per cent by casein, and 30 per cent by lard. Salt mixture and cod-liver oil were mixed with the food; greens were given once a week, and 2 to 3 g. of bread (80–85 per cent extraction) daily. *p*-dimethylaminoazobenzene was added to the food in a concentration of 0·07 per cent; the diet was prepared in quantities sufficient for about 10 days. Food and water were given *ad lib.* It had been found in earlier experiments that on this regime 90 to 100 per cent of the rats developed tumours within 6 to 10 months. In the present series 10 male rats which served as controls received no other treatment. Twenty male rats were treated with soft radiation, and 10 male and 10 female rats were given penetrating X-rays. The irradiations were begun on the same day as the feeding of *p*-dimethylaminoazobenzene, and they were made in exactly the same manner with hard and soft radiation. At the start each rat received 10 r once weekly. The physical factors of the penetrating radiation were: 180 kV, 0·75 mm. copper, 1 mm. aluminium filter (H.V.L. 1·0 mm. copper), and focal distance 30 cm. The rats received 10 r in the liver, i.e. on the ventral surface of the body. Taking the thickness of the rat as 5 cm. the dorsal surface received some 13 r at each irradiation. With the soft radiation the 10 r were calculated with respect to the dorsal surface of the body, and the liver (ventral surface) received only about 0·3 r per irradiation. Factors for the soft radiation: 45 kV, no added filter, H.V.L. 0·33 mm. aluminium, and focal distance 20 cm. Each rat was irradiated individually, in a wooden box in the

case of the hard, and in a celluloid tube in the case of the soft radiation. During the course of weekly irradiations the rats receiving hard radiation lost weight considerably; those receiving soft radiation merely stopped growing. The intervals between the irradiations were then lengthened to 2 weeks, and all the rats resumed approximately normal growth. This fortnightly irradiation was kept up during the greater part of the experiment, but intervals of 3 and 4 weeks were also employed. Over the whole experimental period of 76 weeks the rats were irradiated 7 times at intervals of 1 week, 16 times at intervals of 2 weeks, 4 times at 3-weekly and 4 times at 4-weekly intervals. Thus the rats treated with hard radiation received on the skin of the back about 400 r, in the liver 310 r; the volume doses (calculated for 250 g. rats) were about 100,000 g. $\times$ *r*. The corresponding doses for the rats treated with soft radiation were: 310 r in the skin of the back, 10 r in the liver, and a volume dose of about 24,000 g. $\times$ r.

Two rats in each irradiated group and one control rat died during the first three weeks of the experiment and are not considered further.

RESULTS.

No palpable tumours occurred up to the 8th month. All rats were therefore subjected to laparotomy under ether anaesthesia and the livers inspected. Three of the control and 4 of the hard irradiated rats showed macroscopic tumours. Two irradiated rats had cirrhosis of the liver. No macroscopic changes were observed in the livers of the rats treated with soft X-rays. From the 8th month onward tumours were palpable in some of the control and hard irradiated rats. The experiment was terminated after 76 weeks. At post-mortem the organs were weighed and fixed in formol-alcohol for histological investigation.

Effect on tumour formation.

Table I summarizes the data concerning the occurrence and the rate of growth of the tumours. Large (2 to 10 g.) and very large (up to 50 g.) tumours have

TABLE I.—*Number and Size of Liver Tumours in Irradiated and Control Rats fed* p-*dimethylaminoazobenzene.*

	Number of rats.	Large and very large tumours.	Small tumours.	Microscopical tumours or suspicious changes.	No tumours.
Control, no irradiation	9	5	3	1	0
Soft irradiation	18	1	3	7	7
Hard irradiation	18	7	4	4	3

been grouped together, the macroscopically visible tumours up to pea size form a second group, and tumours of microscopic size or histological changes suggestive of commencing tumour formation in a third; livers devoid of neoplastic change form the fourth group. It is evident that irradiation with soft X-rays delayed and to some extent prevented the formation of tumours. Seventy-seven per cent of these rats had either normal livers or livers showing only incipient proliferative change. No tumour of other origin was found.

In the control group all animals had hepatic tumours. In one rat a pituitary tumour was found in addition (Fig. 1). Microscopically this tumour was cystic,

TABLE II.—*Organ Weights as Percentages of Body Weights in Irradiated and Control Rats fed* p-*mdiethylaminoazobenzene.*

	Number and sex of rats.	Body weight (g.).		Liver.* (g./100 g.).		Spleen (g./100 g.).		Kidneys (g./100 g.).		Gonads (g./100 g.).	
		Range.	Mean.	Range.	Mean.	Range.	Mean.	Range.	Mean.	Range.	Mean.
Control	9 ♂	285–425	353	3·86–7·15	5·04(8)	0·35–1·38	0·53	0·74–1·03	0·88	0·85–1·07	0·98
Soft X-ray	18 ♂	220–450	328	3·00–5·74	4·95	0·25–0·64	0·52	0·67–1·01	0·88	0·56–1·40	1·01
Hard X-ray	10 ♂	185–437	336	3·08–5·25	4·89(9)	0·29–0·92	0·44	0·74–0·97	0·88	0·58–1·22	1·00
Hard X-ray	8 ♀	200–325	235	4·63–7·53	5·30(6)	0·44–0·98	0·73	0·71–1·11	0·98	0·031–0·113	0·051

	Number and sex of rats.	Thymus (mg./100 g.).		Adrenals (mg./100 g.).		P.	Pituitary mg./100 g.		P.
		Range.	Mean.	Range.	Mean.		Range.	Mean.	
Control	9 ♂	31·8–55·9	44·6	11·6–17·0	12·9	—	2·0–3·1	2·4	—
Soft X-ray	18 ♂	43·8–54·7	47·7	13·0–25·0	16·4	$<0·01$	2·2–4·5	3·3	0·1–0·05
Hard X-ray	10 ♂	19·0–56·3	30·2	6·9–23·9	15·7	0·2–0·1	2·5–5·0	3·1	0·1–0·05
Hard X-ray	8 ♀	—	—	25·2–47·0	36·6	—	6·1–7·0	6·9	—

* Livers in which large tumour masses could not be separated from liver tissue are excluded. The figures in brackets represent the number of animals in these groups.

resembling a haemangioma ; it is impossible to say whether it was due to the *p*-dimethylaminoazobenzene.

In the group receiving hard X-rays the number of animals with very small tumours and those not developing tumours was increased as compared with the controls, but the small number of rats and the circumstance that rats of both sex were included in this group does not permit any definite conclusions. In two of these rats (both males) cutaneous tumours occurred. In one of them two primary cutaneous tumours developed, one being a slowly growing epithelioma of the back, whilst the other, which appeared near the hind leg about 3 months later, grew rapidly and consisted of solid columns of non-keratinizing epithelial cells ; microscopically it was quite different from the epithelioma (Fig. 4, 5). No hepatic tumour was found in this rat. The second rat developed a strongly keratinizing epithelioma near the right axilla. Underlying this tumour hypertrophic and cystic glandular tissue was found. The liver of this rat had several small tumours. No cutaneous tumour has ever arisen spontaneously in any of our stock rats.

Histologically the tumours in the liver were mostly hepatomas and non-cystic and cystic cholangiomas. Hepatomas and cholangiomas were often found in the same liver. No histological differences could be observed between the tumours which developed in the irradiated and in the control group, but in the healthy parts of the livers of irradiated rats there were in several cases groups of cells which were enlarged, their cytoplasm more darkly stained and their nuclei rather dark and bulbous. In many of the livers from the hard X-rayed animals an increase of the connective tissue and cirrhotic changes were more pronounced than in the controls. The livers of most of the rats irradiated with soft X-rays showed fatty infiltration and some patches where the cells were of the signet ring type. In many cases the Kupffer cells were prominent and filled with pigment (Fig. 2).

Effects on other organs.

The changes in the weights of the organs in the three groups of rats are summarized in Table II.

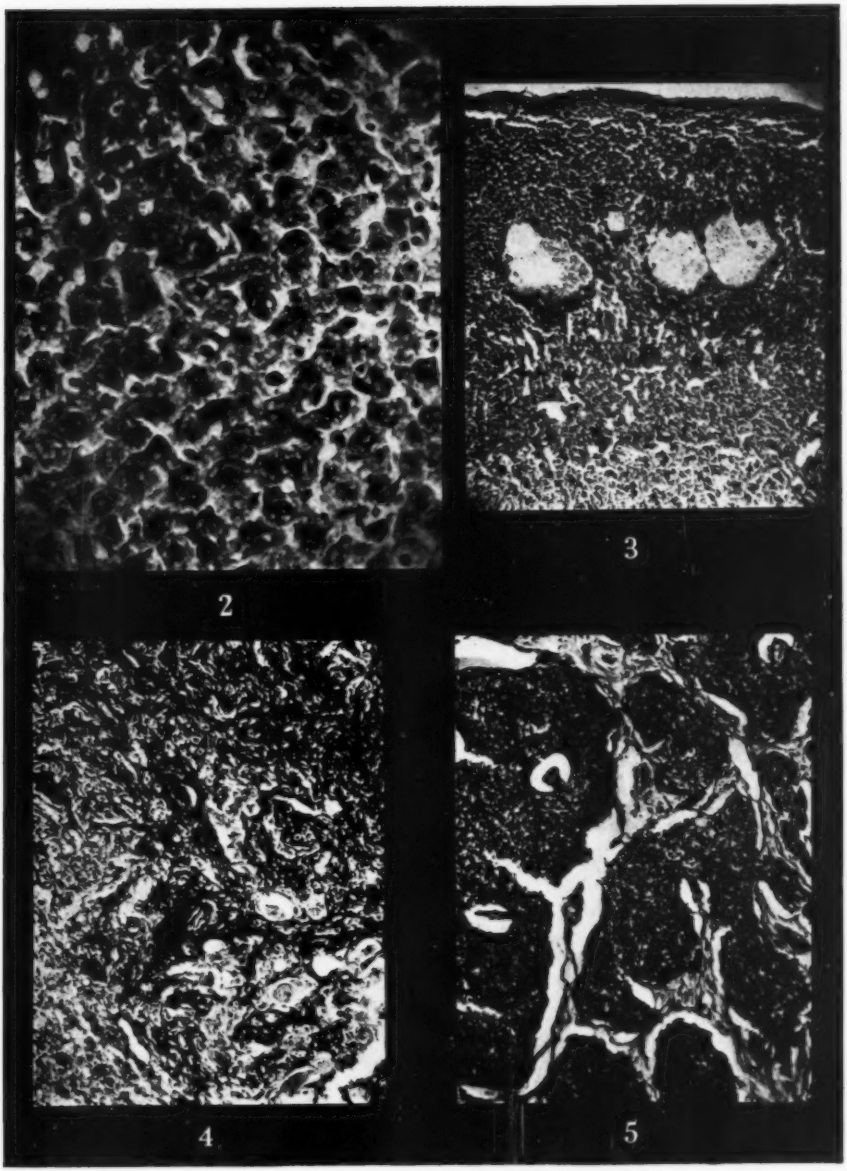
The adrenals in animals of both irradiated groups were enlarged, but this change was statistically significant only in the case of those rats irradiated with soft rays. Whether the difference in enlargement of the adrenals in the two irradiated groups is real is difficult to decide, as only the male rats of the hard irradiated group could be used for this comparison ; female rats have always larger adrenals than males. In previous experiments (Hoch-Ligeti, 1947, 1948) on the effect of the administration of small doses of penetrating and soft X-rays on healthy rats it was found that the increases in adrenal weights of male rats were significant in both cases.

The adrenals of the rats receiving the soft X-rays showed microscopically an increase of the cortex, mostly of the zona reticularis, but also of the

EXPLANATION OF PLATES.

FIG. 1.—Tumour of the pituitary in a control rat fed *p*-dimethylaminoazobenzene.
FIG. 2.—Kupffer cells filled with pigment in the liver of a rat irradiated with soft X-rays. (× 180.)
FIG. 3.—Multiple cysts in the adrenal cortex of a rat irradiated with hard X-rays. (× 65.)
FIGS. 4 and 5.—Cutaneous tumours in a rat irradiated with hard X-rays. 4 = epithelioma, 5 = columns of non-keratinizing epithelial cells. (× 95.)

Hoch-Ligeti.



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Hoch-Ligeti.

fasciculata. In many cases large amounts of pigment were deposited in the reticularis. The cells of the zona fasciculata were larger, pale staining, and sometimes contained large amounts of fatty or lipoid material. In some cases in both fasciculata and glomerulosa nests of large, deeply staining cells were present. In some adrenals incipient cysts were found.

Very similar changes were found in the adrenals of the rats irradiated with hard X-rays, but the formation of cysts was much more pronounced. In 12 out of 18 pairs of adrenals single or multiple cysts of various sizes were found in the cortex (Fig. 3).

Similar cysts, sometimes visible to the naked eye as small red spots, were observed in the adrenals of the control rats, but the other changes seen in the irradiated animals were not found.

In both irradiated groups the weight of the pituitaries was increased, but this increase was too small to be significant statistically with the small number of animals.

The weights of the other organs did not differ in the irradiated and control groups. In particular there was no decrease in the weight of the spleen, thymus or gonads of the irradiated rats. No characteristic change could be demonstrated in the appearance of the thymus of these rats in routine histological examination, nor were any signs of lymphatic atrophy found in the spleens; on the contrary, in many cases the spleen of the soft-irradiated rats showed cellular haloes around enlarged Malpighian bodies, suggesting hypertrophy of the lymphoid and epithelioid elements. The testes, which appeared histologically normal in the control group, showed in 6 out of 10 hard-irradiated rats degeneration of the tubular epithelium with absent or greatly reduced spermatogenesis. Reduced spermatogenesis was found in 3 soft-irradiated rats, and testicular atrophy in one. In the female rats irradiated with hard X-rays the ovaries contained numerous big luteinized follicles; Graafian follicles were absent, except in one ovary. Two uteri showed cystic hypertrophy of the dilated glands.

DISCUSSION.

It has been established (Packard, 1932; Langendorff and Reuss, 1933) that the biological effect of ionizing radiation is independent of the wave-length. The different effect of soft and hard X-rays found in the present work may be due, therefore, either to a difference in the volume doses received by the two groups of rats or to differences in penetration, i.e. to a difference in the organs directly radiated. If it is assumed that a quantitative difference in the volume dose is the determining factor, it would follow that a certain system or systems involved in the mechanism or tumour development are activated by the dose delivered by soft radiation, but may be damaged in their function by the dose delivered by the hard radiation. If the difference in penetration is the determining factor, it is remarkable that in the experiment with hard radiation in which the liver received 310 r of direct radiation, no definite protection against carcinogenesis by *p*-dimethylaminoazobenzene was found, yet with soft radiation where only a negligible quantity of rays could have affected the liver directly, the livers were protected against the effect of *p*-dimethylaminoazobenzene. It appears, therefore, that radiation may have some indirect effect on tumour formation.

It has been suggested that the state of the reticulo-endothelial system is a decisive factor in tumour development. The vast amount of work and discussion bearing on this question has been reviewed by Stern and Willheim (1943). Relevant work was published recently by Stern (1948) and Davidsohn and Stern (1949). The findings in the present experiment are not contradictory to the assumption that the reticulo-endothelial system is affected by the radiation. Dougherty, White and Chase (1944) and Dougherty and White (1945) consider that the lymphoid organs and the state of immunity is dependent on the secretions of the adrenals and the pituitary. In the work here presented soft radiation produced a significant increase in the weight of the adrenals and a slight increase in the weight of the pituitary. The histological changes of these adrenals are consistent with a hyperfunctional hypertrophy. The cystic changes found in the adrenal cortex of the hard-irradiated rats could be interpreted as a sign of degeneration after excessive stimulation. Changes suggestive of a direct activation of the cells of the reticulo-endothelial system are the large increase of Kupffer cells in the liver and the hypertrophy of the epithelioid elements in the spleen of the irradiated rats which did not develop tumours.

The question of an indirect effect of radiation on the body generally when X-rays are used locally to destroy tumours was reinvestigated by Kaplan and Murphy (1949). They found that in mice the dose used locally for the destruction of malignant cells might adversely affect the body defences against the development of metastases. Similar conclusions were reached by Russ, Chambers, Scott and Mottram (1919) on theoretical grounds.

Apart from one subcutaneous sarcoma which occurred after injection of very large doses of *p*-dimethylaminoazobenzene (Kinosita, 1937), this substance has never been shown to provoke cutaneous tumours, nor are X-rays carcinogenic for the rat skin unless previously irritated either by chemical carcinogens or inflammation. The development of skin tumours in the present experiment suggests that the feeding of *p*-dimethylaminoazobenzene sensitizes the skin to the carcinogenic action of X-rays. Experiments on a larger scale are in progress to extend the observations reported.

SUMMARY.

Groups of rats on a diet containing *p*-dimethylaminoazobenzene underwent total body irradiation with small doses of soft or penetrating X-rays.

Whilst all non-irradiated rats receiving *p*-dimethylaminoazobenzene developed hepatic tumours, the development of tumours was prevented, or their rate of growth retarded, in rats treated with soft radiation.

The growth of hepatic tumours was slightly retarded in rats treated with penetrating rays. Skin tumours developed in 2 of these rats.

The adrenals of rats receiving soft X-rays were significantly enlarged.

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SURVEY OF PAPERS.

HARNET has made a statistical analysis of 955 cases of cancer of the cervix uteri and 321 cases of cancer of the corpus uteri with regard to civil state, age, parity, history and methods and results of treatment (p. 433).

CLEMMESEN examines the recent literature on the heredity of human cancer, with particular regard to the incidence of mammary cancer and cancer of other sites among relatives of patients with carcinoma of the breast. He stresses the necessity of allowing for difference in the social distribution of cancer and the importance of cancer registration in such genetical studies (p. 474).

CHRISTENSEN, KLÆR AND WINBLAD describe two cases of meningeal tumours with extracranial metastases and compare them with 5 similar cases from the literature (p. 485).

PULLINGER describes spontaneous and induced foci of squamous differentiation in mouse mammae, and discusses their origin and their relationship to squamous-celled carcinomas (p. 494).

LASNITZKI finds that urethane causes a decrease in growth and mitotic activity in cultures of normal tissues, whereas it stimulates growth and mitotic activity in cultures of tumour tissue. It is suggested that the surface activity of urethane may play a role in the growth stimulation of tumour cells (p. 501).

WOODHOUSE finds that the guanine content of the fibrous deoxynucleic acid from mouse and rat sarcoma tissue is greater than that of calf thymus or rat liver nuclear nucleic acid, and that the amounts of thymine and guanine in nucleic acids are not necessarily equi-molar (p. 510).

DMOCHOWSKI AND ORR find that the mammary tumour agent is present in breast tumours induced in C3H high-breast-cancer mice but have failed to find the agent in breast cancer induced in C57 Black and IF low-breast-cancer mice (p. 520).

DMOCHOWSKI gives data on the distribution of the mammary tumour agent in C3H high-breast-cancer strain mice, and also finds that milk in the stomach of 5-day-old C3H high-cancer strain mice is a good source of the agent (p. 525).

HALL AND BIELSCHOWSKY find that the preliminary administration of acetylaminofluorene to rats treated with methyl-thiouracil accelerates the appearance of benign multiple adenomata of the thyroid but does not hasten the development of malignancy (p. 534).

BIELSCHOWSKY, GRIESBACH, HALL, KENNEDY AND PURVES find that thyroid tumours induced in rats by methylthiouracil alone and in combination with acetamidofluorene show no difference in their transplantability (p. 541).

BIELSCHOWSKY finds that adenomata of the thyroid can be induced by acetylaminofluorene in partially-thyroidectomized rats but not in rats with intact thyroids (p. 547).

RASK-NIELSEN has injected 9-10-dimethyl-1:2-benzanthracene subcutaneously in various sites in Street mice, and has obtained isolated thymic tumours and generalized leukaemias in equal numbers in 11·6 to 14·3 per cent of males irrespective of site of injection whereas in female mice he has obtained a total incidence of leukaemia of 21 to 26·5 per cent with a higher proportion of generalized leukaemia after injection in the mammary region (p. 549).

COOK AND SCHOENTHAL find that a large supplement in the diet of choline has no effect on the induction of tumours in rats by 1:2:5:6-dibenzanthracene (p. 557).

MEDUSKI finds that injections of 1:2:5:6-dibenzanthracene in rats cause an increase in glutathione in the liver but have no effect on the ascorbic acid content, and that there is no relationship between the ascorbic acid and glutathione content of tumour tissue and liver in individual animals (p. 559).

HOCH-LIGETI finds that total body irradiation with small doses of soft X-rays prevents the development or retards the growth of hepatic tumours in rats fed on a diet containing *p*-dimethylaminoazobenzene, whereas similar treatment with penetrating X-rays only slightly retards the growth of hepatic tumours (p. 562).